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THE UNIVERSITY OF ALBERTA
ELECTROPHYSIOLOGICAL STUDIES OF OPIATE RECEPTORS
ON FROG SKELETAL MUSCLE

by



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ABSTRACT

Many CNS depressants block excitability by inhibiting the specific increase of Na^+ conductance which normally follows depolarization of the membrane. Some drugs like general anesthetics (steroid anesthetics excluded), act pharmacologically by altering membrane function in a diffuse, nonspecific fashion, as is suggested by the lack of rigorous structural requirements for their actions. There is also evidence to suggest that other depressants like the opiates, act at cells that contain specific 'receptors' for these drugs. The purpose of the present investigation was to investigate the nature, specificity and sensitivity of the opiate receptor on frog sartorius muscle by studying the interactions of a variety of opiates and other depressants on these proposed opiate receptors. Isolated sartorius muscles of Rana pipiens were used to investigate the effects of morphine, methadone, propoxyphene, meperidine, naloxone, naltrexone, dextromethorphan, tetrodotoxin and procaine on certain features of the active muscle membrane, using extracellular electrode and intracellular microelectrode techniques.

Studies with extracellular electrodes showed that application of all the aforementioned drugs decreased both the amplitude of the compound action potential and the excitability of the muscle fibres. With all the drugs, the depressed responses were reversed to the control levels when the preparation was returned to Ringer's solution.

The maximum rate of rise of the action potential was decreased by all the opiate agonists. This effect was antagonized for all the opiate agonists by having present concurrently in the bathing medium, low concentrations of naloxone or naltrexone. The resting potential

remained essentially unchanged in the drug-tested muscles.

Opiate antagonists did not antagonize the non-specific action potential depression produced by dextromethorphan, procaine, or tetrodotoxin.

Subsequent studies with intracellular microelectrodes showed that methadone, morphine and propoxyphene HCl block action potential production by two mechanisms, i.e. there is a biphasic time course for the decline in the maximum rate of rise of the action potentials:

(i) a non-specific mechanism in which the increase in sodium conductance (\bar{g}_{Na}) and in potassium conductance (\bar{g}_K) are depressed and (ii) an opiate drug receptor-mediated mechanism, causing a specific depression of \bar{g}_{Na} . Low, antagonistic concentrations of opiate antagonists could antagonize only the effects produced by the second mechanism (ii).

An explanation for the time course of the second phase of the biphasic depression, is that the receptor sites for the opiates under investigation are located on the inner surface of the plasma membrane and that the opiate molecules attached to these receptor sites are in equilibrium with the opiate concentration in the sarcoplasm.

Higher agonistic naloxone and naltrexone concentrations ($\sim 10^{-3}M$) inhibited the maximum rate of rise in a monophasic fashion. Only the depressant effect of naloxone could be antagonized by concomitantly employing antagonistic concentrations of naltrexone.

External sodium acted in a competitive fashion to antagonize the action potential depression produced by the drugs studied in this study. This investigation has furnished evidence that opiates exert their effects on frog sartorius muscle via interaction with opiate

receptors. The results also suggest that in this system, naloxone behaves as a partial agonist with a low intrinsic activity. However, this does not invalidate the concept that most CNS depressants act by inhibiting the sodium conductivity because this inhibition could occur as a consequence of or subsequent to the interaction of opiates with their "receptors".

The overall impression gained from the results of this investigation is that there are opiate drug receptors located on the inner surface of the muscle membrane associated with the "sodium channels" and that drug activation of these receptors interferes with the opening of the "sodium channels" normally produced by sufficient membrane depolarization.

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I. INTRODUCTION

To understand the cellular mechanisms underlying excitability depression in skeletal muscle produced by central nervous system (CNS) depressants it is necessary to have an understanding of the events that determine muscle impulse initiation and propagation. Hence this introduction focuses first on muscle cellular electrophysiology and second discusses those pertinent reports that have led to our present concepts of the electrophysiological mechanisms of action of some CNS depressants.

The Resting Potential

The cell membrane which exhibits differential permeability (1) is relatively impermeable to most organic compounds (particularly those of high molecular weight, e.g. proteins) and certain other inorganic ions. This differential permeability leads to a characteristic ion distribution; the inside of the cell contains a high concentration of proteins (which cannot readily get out of the cell because of their large size) and potassium (K^+). The extracellular space, contains a high concentration of sodium (Na^+) and chloride (Cl^-) ions (2, 3). This particular distribution of K^+ and Na^+ is due, in part, to the differential permeability of the cell membrane and in part to the presence in the membrane of machinery (e.g. the Na-K pump) for actively transporting ions across the membrane (4, 5).

The ion distribution just described and the properties of the membrane itself produce an electrochemical gradient across the cell membrane. This electrochemical gradient creates an electrical potential difference between the inside and outside of the cell. This potential is called the Resting Potential. It is approximately 85-95 mV for frog skeletal muscle, with the inside of the cell electrically negative with

respect to the outside. This transmembrane potential is largely the result of a potassium ion concentration gradient maintained across the cell membrane by active transport involving the enzyme sodium-potassium activated ATPase.

The resting membrane has a high electrical resistance (6) and is relatively impermeable to sodium and potassium ions. Since its low ionic permeability is mainly accounted for by transmembrane movement of K^+ and Cl^- , the resting membrane potential is relatively close to the equilibrium potential for these ions (3, 7).

The Action Potential

Muscle cells and neurons exhibit additional properties that cause them to be referred to as "excitable". The resting potential of such cells is "unstable" under certain conditions. If such a cell is made to depolarize (resting membrane potential decreased towards zero) to a certain potential level, the so-called 'threshold potential', a rapid phase of depolarization occurs that ultimately reverses the electrical potential across the cell membrane so that momentarily the interior of the cell becomes positively charged with respect to the exterior surface of the cell membrane. At the peak of this event, the interior of the cell has a positive electrical potential of approximately 40 mV as compared to the exterior of the cell in skeletal muscle fibres. This is largely a passive process, because of the presence of transmembrane electrical and concentration gradients. Degrees of depolarization lesser than the threshold potential result only in transient displacement of membrane potential and not in an action potential. After completion of the depolarization phase, repolarization begins and during this time, the electrical potential within the cell again

becomes progressively more negative with respect to the exterior of the cell until such time as the resting potential is re-established (9, 10). This whole phenomenon (i.e. depolarization and then repolarization) is called the Action Potential and it is unique to excitable cells.

Once the action potential is initiated, it proceeds "automatically" to completion, independently of the properties of the stimulus that caused the membrane to depolarize. Thus, it is said to occur in an all-or-none fashion. The membrane is inherently refractory for a period of time following an action potential and cannot be made to generate another response during this refractory period. The action potential is propagated over the entire membrane without decrement and at a finite (but high) velocity (8, 11, 12).

At least since the time of Bernstein (1) action potentials have been thought to be associated with transient changes in the structure of excitable membranes that allow an increased movement of ions across these membranes. The rate and direction of ion movements through excitable membranes during an action potential are dependent upon the electrochemical gradients present across the membranes. The ion movements occur in milliseconds during action potentials and the net ion flux per unit of surface area is higher than the resting flux (12). Most investigators, have interpreted data concerning these processes in terms of diffusion ion movements through "channels" or pores rather than in terms of carrier mechanisms (11, 13, 14, 20, 21, 25).

Thus, the current view is that the action potential is generated by voltage-dependent Na-selective and K-selective channels in excitable membranes and that these channels are probably protein molecules imbedded in the membrane whose configuration is dependent on the

voltage across the membrane. It is in this manner that the membrane's ability to allow certain ions to pass through is altered by changes in the membrane potential at any instant in time (11, 14, 21). Sodium channels are probably composed of lipoproteins that span the thickness of the nerve membrane (234). The inner end of the sodium channel is thought to be the location of structures that open and close the channel, the so-called "gating functions" (235). Studies with certain toxins have given rise to the contention that the resting sodium channel and the active sodium channel are distinct entities (236).

Upon excitation there is an initial increase in the permeability of the cell membrane to sodium ions that results in an influx of sodium ions from the exterior of the cell membrane to the interior of the excitable cell. The influx of Na^+ discharges the membrane capacity and so increases depolarization, with a consequent further increase in sodium permeability (PNa). If a compensating outward current of K^+ cannot prevent substantial depolarization, the entry of Na^+ becomes self-regenerative and within a millisecond the potential reaches a peak of positivity, approaching the equilibrium level for Na^+ . This inward movement of Na^+ accounts to a large extent for the upstroke or the depolarization phase of the action potential (12).

During the rising phase of the action potential, as it approaches its peak, the permeability of the cell membrane to Na^+ decreases and at about the same time but independently, the membrane permeability to potassium ions increases above its resting level. These two events combine to bring the membrane potential back to the resting level rapidly. The number of ions transported during one action potential is too small to modify measurably the concentration difference across the membrane.

The spike draws current from adjacent resting areas of excitable membrane, exciting them and so once initiated, the spike propagates itself to the ends of the fibre from the point of initiation. The movement of Na^+ and K^+ ions across the cell membrane during the period of excitation is a passive phenomenon, since both ions are moving down their concentration gradients (11, 12). Immediately after repolarization there is a slight excess of sodium ions and a slight deficiency of potassium ions within the cell. Although the excitation process has been completed and the cell is electrically quiescent, sodium ions must be extruded from the interior of the cell against a concentration gradient. This can only be accomplished by an active transport process (sodium pump) requiring energy. The energy required to drive the so-called sodium pump is derived from the oxidative metabolism of adenosine triphosphate (ATP). It is believed that the metabolic pump responsible for the extrusion of intracellular sodium ions is also responsible for the transport of potassium ions from the extracellular space to the interior of the cell to restore the normal $(\text{K})_i/(\text{K})_o$ ratio across the cell membrane (19, 22, 23). However, potassium ions may also return to the interior of the cell down an electrochemical gradient caused by the extrusion of Na^+ ions. This would not require an energy expenditure.

On the basis of the above membrane theory (12) the action potential is explained by a cycle of permeability changes in the membrane.

Action Potential Propagation

The self-propagating nature of the impulse is due to circular current flow and successive electrotonic depolarizations to the firing level of the membrane ahead of the action potential. Once an action potential occurs at a given site, currents flow from this site along

the inside of the fibre and across the membrane. Current travelling outward across the membrane in areas not yet excited reduces membrane potential beyond threshold potential and results in regeneration of the action potential. In a skeletal muscle fibre the impulse moves along the surface in a nondecremental fashion, i.e. at a constant amplitude and with a conduction velocity of 1-10 metres per second. The conduction velocities (velocity of propagation) in excitable cells are determined by several factors, including the radius of the cells, "passive" electrical properties of the membrane (resistance and capacitance), membrane potential, threshold potential and the maximum rate of rise and amplitude of the action potential (237).

Sodium Hypothesis

While studying the effect of sodium ions on the electrical activity of squid giant axon, Hodgkin and Katz (219) demonstrated that: (a) replacement of external Na^+ by sucrose or choline reversibly reduced the size of the action potential and abolished excitation; (b) height of the overshoot of the action potential varied linearly with the logarithm of the external sodium concentration; and (c) a reduction in sodium concentration caused a reversible decrease in the rate of rise of the action potential.

Nastuk and Hodgkin (8) and Desmedt (15) have confirmed the validity of the sodium hypothesis for the sartorius muscle of the frog. Their results indicate that the magnitude of the action potential and overshoot of the spike are also linearly proportional to the logarithm of the sodium concentration in the external fluid.

Hodgkin and Katz (219) predicted that the rate of rise of the action potential should be determined by the rate of entrance of sodium

into the membrane and to rough approximation, the rate of rise should be directly proportional to the external concentration of sodium. Recently it has been found that about 1% of this 'sodium current' in squid axon may be due to the entry of calcium ions (24). The experimental findings of Nastuk and Hodgkin (8) and Desmedt (15) with frog skeletal muscle showed that the rate of rise of the action potential is likewise influenced by the sodium concentration in the bathing medium. This is clearly consistent with the idea that the active depolarization of a muscle fibre is due to entry of sodium.

Although the action potential of excitable tissues is invariably accompanied by an increase in the conductivity of the membrane, it does not always depend on an influx of sodium in all tissues (17, 18).

II. EFFECTS OF ANESTHETIC DRUGS ON THE NERVOUS SYSTEM

"... an anesthetic is not a special poison for the nervous system. It anesthetizes all the cells, benumbing all the tissues and stopping temporarily their irritability ... We can study elsewhere than in the central nerve cells, the phenomenon which causes this stoppage of action and ... It is permissible to assume that something similar happens in the nerve cell."

CLAUDE-BERNARD
Lecons sur les anesthesiques
et sur l'asphyxie (1875)

An important implication of the sodium hypothesis, at least from the pharmacological standpoint, is that any agent which interferes with the specific increase in sodium permeability of the excitable system, will lead to an impairment of excitability in this system.

The 'Excitability or Permeability Theory of Narcosis' (26), states that in the presence of anesthetic drugs, the cell membrane is modified in such a manner that stimulation can not produce the normal rapid increase in permeability required for the ionic exchanges producing the action potential. Thus, anesthetic drugs produce central nervous system (CNS) depression because they interfere with the movement of ions necessary for the transmission of the nervous impulses.

The most general definition of an anesthetic (or more properly a local anesthetic) is a drug which when applied directly to the nerve or muscle cell, blocks the action potential in a reversible manner without appreciably affecting the resting membrane potential of the cell (27, 28). According to this definition, a wide variety of

lipophilic compounds are anesthetics (i.e. block the sodium conductance channel): local anesthetics (42, 44, 58), barbiturates (31, 238), tranquilizers (58, 239, 240), alcohols (57, 241), anticonvulsants (242, 243, 244), antihistamines, steroids, detergents, antiarrhythmics, vasodilators (67) and opiates (56, 194). There also exist irreversible anesthetics, such as the haloalkylamines, which give sustained anesthetic action on nerves.

It continues to be generally assumed that the primary actions of anesthetics are on the cell membrane (plasmalemma) rather than on intracellular processes (29). This assumption is warranted by three types of observations:

- (a) Perfused axons, devoid of their axoplasm are still readily blocked by anesthetics (30, 31).
- (b) Nerve block and synaptic block occur at concentrations of anesthetic, which are lower than those required to inhibit metabolism and oxygen consumption (32).
- (c) Action potentials in artificial lipid bilayers are blocked by anesthetics such as cocaine or chlorpromazine (33).

Other studies, however, have provided some evidence indicating that the primary anesthetic action is on the membranes of intracellular organelles such as microtubules (34, 35, 36) or mitochondria (37). These intracellular actions, however, are obviously more difficult to study and also sometimes occur at anesthetic concentrations which are usually 5 to 20 times higher than those required to block the action potential (38, 39, 40).

Cole and Curtis (6) demonstrated that membrane resistance decreased during electrical activity and that the blockade of excitability by most cellular depressants was due to a reduction in the normal increase in permeability which is responsible for the upstroke of the action potential.

Straub (41) suggested that local anesthetics depress the increase in membrane sodium conductance which occurs simultaneously with, and is responsible for the rising phase of the action potential in nerve fibres. Also in 1956, as a consequence of his study of several general anesthetics on intact frogs and on excitability processes in frog's skeletal muscle, Thesleff (27) proposed the concept that General Anesthetics produce their CNS effect by inhibiting the specific increase in sodium conductivity responsible for action potential production in excitable cells. He further demonstrated that the concentrations of the various anesthetics required to depress excitability in frog's skeletal muscle fibres were well correlated with the doses required to produce general CNS depression in the frog.

Taylor (42) and Shanes et al. (43) employing local anesthetics on the squid giant axon, obtained evidence supporting the mechanism of excitability blockade suggested by Straub (41). Subsequently, when it was discovered that local anesthetics had the same mechanism of action on frog's skeletal muscle fibres it was proposed by Frank and Sanders (47) and Frank and Jhamandas (245) that all drugs which had this mechanism of action on excitable cells, would be able to produce general CNS depression in intact animals under appropriate conditions.

Thus, a great variety of heterogenous groups of excitability depressants, have one important property in common; i.e., they all

produce a local anesthetic-like effect when applied to excitable cells. In confirmation of this theory, it has been found that drugs which produce local and general anesthesia affect the bioelectrical signals by increasing the threshold for excitation and by decreasing the increase in sodium conductivity of the cell membrane following a stimulus (27, 42, 44, 45, 46, 47, 51, 52, 53, 54). Of particular importance in this regard was the demonstration that tetrodotoxin (TTX) had general CNS depressant properties (53), because tetrodotoxin has been shown to block excitability by a unique mechanism involving a specific drug receptor (378).

Although numerous studies support the anesthetic blockade of the sodium conductance, it has also been shown that they all to some extent also suppress the potassium conductance which is responsible for the repolarization of the cell membrane (42, 44, 55, 56). The anesthetic concentration needed to depress the K^+ conductance of the action potential is generally about ten times higher than that required to depress the Na^+ conductance channel (14, 57, 58, 59).

Electrophysiological studies have demonstrated that anesthetics and sodium ions behave as competitive antagonists (44, 45, 46, 48, 49, 54, 56, 60, 61, 62). Hille (58) suggested that anesthetics decrease the magnitude of the sodium current because they interact with the sodium channels and thus in turn decrease the available number of channels for the sodium ions.

Weidmann (63), Thesleff (27) and Frank (189, 190), have made a parallel suggestion in that anesthetics and other central depressants cause a reduction in the availability of the "sodium carrying" system and thus decrease the conductance of sodium in the active cell membrane.

The evidence presented thus far strongly supports the hypothesis that the prime action of general CNS depressant drugs is to reduce the specific increase of Na^+ conductance in the membrane, the resting membrane potential remaining practically unchanged at blocking concentrations of the anesthetics.

Probably the main reason why investigators have sought so long to demonstrate a single common basic mechanism of action for general CNS depressant drugs is the remarkably similar pattern of CNS depression produced by so many drugs with differing chemical characteristics. Nevertheless all have had to contend with the fact that in addition to producing this common pattern of general CNS depression, these drugs also produce other effects on the CNS which are unique to themselves or to only a few drugs with closely related structures. Although differences in the membrane effects of these drugs have been observed, no attempt has been made to relate these differences to other CNS effects of these drugs.

Opiate Drug Receptors

The concept of selective receptors for drugs, hormones, and neurotransmitters is fundamental to pharmacology. Receptors are thought to be macromolecules, predominantly protein, located generally on the membranes of cells (exceptions exist e.g. steroid receptors) and uniquely affected by the drug, hormone or neurotransmitter. By interacting with receptors, often in minute doses, such agents can exert selective effects. It is assumed that at least a portion of the macromolecular receptor is present on the surface of the plasma membrane. An activating molecule, or agonist, interacts with the activation site of the receptor. The drug-receptor interaction is a dynamic process involving

an interaction by intermolecular forces, mutually molding drug and receptor. Therewith, conformational changes in the receptor molecule are induced that trigger the sequence of biophysical and biochemical events leading to the effect (246). The formation of this agonist-receptor complex in excitable cells then affects conductance changes by activating or altering an ion permeation mechanism.

A complication that arises is that membranes expose a variety of "target" structures with respect to an interaction with drugs. Among the interactions known so far are: (a) Direct or allosteric specific interactions with enzyme proteins (e.g. acetylcholinesterase), with transport proteins (e.g. cardiac glycosides and Na-K-ATPase), with proteins (e.g. tetrodotoxin with Na-pore), and with commonly known receptor proteins (e.g. acetylcholine-receptor). (b) Unspecific interactions with proteins (e.g. of SH-group reactants). (c) Physiochemical interactions with lipids (e.g. anesthetics, barbiturates, neuroleptic agents). (d) Interactions with false constituents (e.g. substitution of cholesterol by desmosterol or diazacholesterol). (e) Alteration of fatty acid chain or lipid pattern by substitutional exchange with different fatty acid residues or lipids. (f) Indirect influence upon membrane properties by alteration of membrane dipole moment through alterations of the transmembrane electric field by a variety of drugs.

More or less any of the interactions mentioned may, because of the intimate functional and structural interrelationship between membrane constituents, spread beyond the 'local event' and involve alterations of a variety of other membrane properties (248).

Although the molecular sites of action form an indispensable

concept in the understanding of drug action, their molecular characteristics such as chemical properties, location and number are usually unknown. To indicate them they are termed receptors. They are located in or on target cells. There is a tight interrelation of receptor molecules and their surrounding. A special problem which holds for all receptors is that with isolated receptors no measurable "pharmacological" effect can be induced anymore, making their identification extremely difficult in certain cases,

However, a difficulty with the above definition of receptors is the situation in which a large number of structurally different chemical substances all appear to act at the same site or sites in the living organism to produce the same drug action. In such cases the existence of specific drug receptors is questionable. This is the situation with respect to the local and general anesthetics and most general CNS depressants. In many respects this whole area is the despair of the pharmacologist, leading some to question the very existence of drug receptors for these drugs (230) and many others simply to ignore the possibility of their existence (67).

In discussing the mode of action of membrane active drugs, an important and central distinction is that between so-called non-specific and specific action. The former-typified by the effects of nearly all anesthetics and many analgesics - appears to be basically physical, depending entirely on the drug's capacity to enter the membrane structure. The compound must be lipid soluble, but otherwise its chemical structure appears to be essentially irrelevant. In specific action, by contrast, chemical structure is critical. In the opiate narcotics for example, only the levorotatory forms are

pharmacologically active; the mirror-image dextrorotatory forms have little or no effect, though their lipid solubility and other physical properties (apart, of course from their optical activity) are identical. Specific action, though not yet well understood, probably involves the triggering by the drug molecule of a distinctive chemical receptor on the membrane surface, which in turn can set off changes within the cell.

Of the many fundamental actions of psychoactive drugs on membranes (67) there are four of outstanding importance. These are: (a) Membrane Expansion, (b) Membrane Fluidization, (c) Effects on Membrane Calcium, and (d) Blocking of Ionic Channels (e.g. TTX).

All the lipid soluble nerve blocking drugs are effective in expanding membranes (220). The general evidence available indicates that membrane expansion results in a distortion of the Na^+ conductance channel, responsible for the nerve impulse, that is to say that membrane expansion produces an electrical stabilization of the membrane. However, this is by no means to say that they are reacting exclusively with the proteins. On the contrary, they may also alter the properties of membrane lipids and it is these lipid interactions that probably account for many of the clinically significant differences between various anesthetics, analgesics, and other centrally acting drugs.

A number of experiments have established that the membrane expansion induced by nonspecific drugs is accompanied by an increase in lipid fluidity (229). Perhaps an important effect of this increased fluidity, at least so far as the CNS is concerned, is that it apparently facilitates the release of neurohumors (379). And there is

reason to believe that the more fluid the membrane, the more readily the vesicles fuse with it. Thus the capacity of the nonspecific drugs to facilitate nerve discharge, by their fluidizing of membrane lipids, as well as to inhibit it, by blocking the protein sodium channels, makes for all sorts of complications.

Another complexity of nonspecific drug action concerns membrane calcium. The psychoactive drugs absorb to the membrane in very high concentrations and by this means displace the membrane bound Ca^{++} (67). The neutrally charged psychoactive drugs, however, either have no effect on the membrane bound Ca^{++} or cause it to increase. Ca^{++} is an obligatory requirement for excitation-contraction coupling (249). It is required for stimulus secretion coupling and may thus modulate the release of neurotransmitters (204, 205) and also affect neurotransmitter receptor interactions (250, 251). Ca^{++} plays a role in membrane stabilization (206, 207) and is probably involved in the initiation and or conduction of nerve impulses (208, 209, 210) and in the post-synaptic action of neurotransmitters (211, 212, 213). Ca^{++} is also known at least in vitro to alter the activity of many enzymes (tryptophan and tyrosine hydroxylase as well as adenylyl and guanylyl cyclase) of the nerve cell (214, 215, 252). Certain classes of nonspecific drugs e.g. tertiary amines (examples are procaine, chlorpromazine) displace Ca^{++} from the membrane whilst the acidic anesthetics (e.g. barbiturates) increase binding of Ca^{++} to membrane lipid. There is considerable evidence that calcium is involved in many functions, many of which are also directly or indirectly affected by opiate treatment (253). Thus, Ca^{++} could antagonize the effects of morphine by alteration of one or more of these aspects of nerve function, or

by direct competition with morphine for the narcotic receptor as has been suggested by several in vitro studies (110, 138, 216, 253).

Harris et al. (202, 203, 217) found that Ca^{++} , Mg^{++} and Mn^{++} could antagonize the antinociceptive effects of morphine when injected intraventricularly in mice and rats. These ions produced parallel shifts of the morphine dose response curve, possibly indicating a competitive antagonism of morphine. In addition agents which chelate Ca^{++} potentiate the effects of opiate drugs and have been reported to have a weak antinociceptive effect of their own (202). Brain calcium levels are decreased by acute administration of morphine and this effect is blocked by naloxone (254).

These investigations also indicated that elevation of brain Ca^{++} levels antagonized the analgesic effects of morphine. The antagonist actions of Ca^{++} appeared to involve increased penetration of Ca^{++} across cell membranes, because calcium antagonists appeared to block these actions of Ca^{++} . Inasmuch as the effect of various cations on morphine action did not parallel their effects on stimulus-secretion coupling, it would appear that the narcotic antagonist action of Ca^{++} probably is not the result of a presynaptic action. However, a postsynaptic site might be involved in this action as postsynaptic alterations in the binding and movement of Ca^{++} have been shown to be involved with the response to several neurotransmitters (211, 212), and it has recently been proposed (213) that the postsynaptic intracellular Ca^{++} concentration may serve to modulate synaptic transmission.

The results of these and other investigations indicate that alterations in the levels of intracellular Ca^{++} , perhaps at a post-

synaptic site, may be closely related to the actions of narcotic agonists and antagonists. Recent reports indicate that lanthanum, a well known Ca^{++} antagonist may have a neuroanatomical site of action similar to morphine in producing analgesia and in fact can substitute for morphine in a cross-tolerance situation in producing analgesia (255, 256, 257).

Given all these complexities, beginning with the size of the drug molecule, continuing with its relative affinity for various membrane constituents, and concluding with its capacity to bind Ca^{++} to or release it from the membrane, the possible variations in terms of drug action of a given compound become virtually infinite.

This difficulty is further compounded when one notes that specific and nonspecific are not mutually exclusive terms. Certain drugs appear to act in a "semispecific" manner, partaking of both modes, while others combine both specific and nonspecific actions, the dominance of one or the other often depending on the concentration of the drug employed.

Drugs that appear to combine both specific and nonspecific effects are the opiates e.g. morphine (56, 194, 258, 259). In low concentrations morphine is specific, producing analgesia only in its l-rotatory form, the d-form being far less potent. In much higher concentrations, however, it appears to obey nonspecific rules, with both forms producing anesthesia. According to a recent report, (65) indeed, a surgical group has been employing massive doses of opiates (around 10 mg/kg) for anesthesia in cardiac surgery. Further high pressure which reverses conduction blocks produced by inhalational anesthetics (260, 261),

ethanol and local anesthetics (262), also reverses general anesthesia elicited by ethanol (263), inhalational agents (264), barbiturates (264, 265), opiates and neuroleptics (264). A high dose of morphine produced tadpole narcosis, which could be reversed by pressure (380). Pressure reversal was also demonstrated in this case for the steroids in althesin.

However, a significant feature of the above findings is that morphine and alphaxalone (a major component of althesin) produce most of their effects via a receptor mediated process, because these effects are antagonized by antagonists such as naloxone and Δ^16 -alphaxalone respectively (56, 266). The problem that arises is whether morphine and alphaxalone produce general anesthesia via a receptor mediated process or through their nonspecific action. Such a nonspecific action of morphine is known to occur at high concentrations (56, 194, 258, 267, 268). In this regard opiate antagonists have been shown to antagonize general anesthesia produced by cyclopropane, halothane (269) and nitrous oxide (270). Thus these investigators concluded that either anesthetics release endogenous analgesics (i.e. all anesthetics may mediate anesthesia via release of endogenous opioid like substances) or else narcotic antagonists have analgesic antagonist properties heretofore unappreciated (271).

It has been suggested (272) that there are three kinds of interaction between an opiate and membranes containing opiate receptors: (i) a non-saturable interaction ("trapped and dissolved") consisting primarily of the physical solution of lipophilic opiate molecules in the lipidic membranes; (ii) a nonspecific saturable binding, consist-

ing primarily of interactions between the protonated nitrogen atom of the opiate and anionic groups of membrane macromolecules (most opiates at physiological pH are cationic); (iii) the stereospecific interaction of D (-) opiates with opiate receptors.

Other factors postulated (273) governing the degree of drug activity are: (i) the rate of penetration of the drug to the receptor site, (ii) a fit factor between the drug and the receptor site, (iii) the rate of reaction or interaction between the drug and some component or components of the receptor site, (iv) the rate of wastage (hydrolytic, oxidative, reductive and nonspecific binding) of the drug on its way to the receptor site. A question that remains unanswered is whether the relationship between the logarithm of the partition coefficient of the drug and its rate of penetration to the receptor site still holds for the iterated process involving transfer of the drug through many cells or across a multiplicity of membranes (273).

Although in most instances the existence of drug receptors for general CNS depressant drugs remains questionable, the evidence for the existence of specific opiate drug receptors is most compelling. This evidence can be summarized as follows:

- (a) Despite some leeway, opiates conform to a particular chemical structure, i.e., there are striking commonalities in the chemical structures of most opiates (Figure 1).
- (b) Some opiates (e.g. etorphine) exert their effects in extremely small doses.
- (c) Some marked stereospecificity of opiate actions

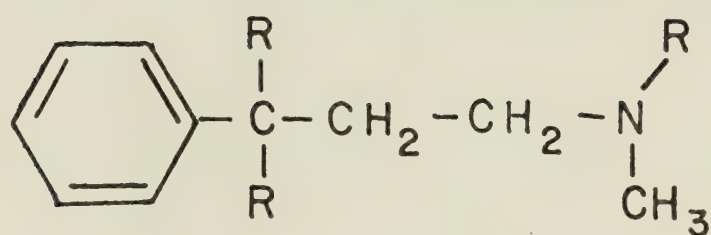


Figure 1: Minimal structural requirements for narcotic analgesic activity (adapted from, 68).

- strongly favor highly selective stereospecific receptor sites. For most opiates the D(-) isomer contains essentially all pharmacological activity of the drug, the L(+) form being virtually inactive.
- (d) The existence of opiate antagonists also argues for a specific opiate receptor.
 - (e) Saturability implying that the number of binding-sites (i.e. "receptors") is limited.
 - (f) The recent discovery (73, 74, 75, 76) of endogenous ligands for the opiate receptors, very strongly argues for the existence of such receptors.

Some Properties of Isolated Opiate Receptors

One approach taken by some investigators, is to study opiate drug binding to entities which they claim represent the "opiate receptors" (77, 78, 79, 80, 81, 82, 83). Creese & Snyder (85), compared the pharmacological and binding potencies of a series of opiate agonists and antagonists in the guinea pig intestine because the ability of opiates to inhibit electrically induced contractions of the guinea pig intestine is well known to parallel closely analgesic activity (84). In all cases examined by Creese & Snyder (85), the concentration of drug occupying half the binding sites corresponded closely to the concentration that produced half of the maximal pharmacological response, whether that be an agonist or an antagonist response. They suggested that, for opiates, pharmacological responses can be explained without invoking "spare-receptors".

Another well known theory holds that many variations in pharmaco-

logical potency of drugs are determined both by the affinity with which the drug binds to the receptor and its "intrinsic activity" to transform receptor binding into pharmacological activity (86, 87, 88, 89). For all the opiates examined by Snyder et al. (100), pharmacological potency corresponded so well to binding potency, that they stated that receptor affinity alone could suffice to account for the potency of opiate agonist drugs.

The molecular structure and configuration of opiate antagonists is virtually identical to that of the opiate agonists. It must be assumed that the antagonists can occupy the same receptor sites as the agonists, and this is consonant with the frequently demonstrated competitive nature of the antagonism (96, 97). It follows that site occupancy of the receptor itself, cannot produce opiate effects. An opiate agonist has to do more than occupy the appropriate receptor site; it has to produce some positive change in the receptor. This change, which could be a conformational alteration, is evidently brought about by an interaction in which the protonated nitrogen atom plays an essential role. According to this view, an antagonist is a compound that occupies the receptor site without causing such a change and thereby prevents agonists from interacting there.

It was discovered that sodium enhances receptor binding of antagonists, but decreases the binding of agonists to "isolated" opiate receptors (98, 99). Conversely high manganese ion concentrations were shown to enhance agonist binding (274). Snyder et al. (99) postulated that the isolated opiate receptor can assume different conformations: one in the presence of sodium which binds antagonists more efficiently

and another in the absence of sodium, which binds agonists more efficiently. Presumably sodium allosterically, transforms the isolated 'receptor' between the two conformations (99).

Since opiate agonists and antagonists are similar chemically and compete with each other for receptor binding, Snyder et al. (100) postulated that both drugs bind to the same receptor, but that the opiate binding site can vary as the receptor is transformed between two conformations (99, 101).

The interconversion of the two forms of the isolated opiate receptor presumably involves folding, unfolding, aggregation, disaggregation or other modifications in protein structure, since the action of proteolytic enzymes on receptor binding (101) indicates that the opiate receptor is proteinaceous and contains phospholipid components.

Since the opiate receptor would normally be largely in the antagonist state, agents interfering with the interconversion of receptor conformations would selectively reduce agonist binding (100). Consistent with these predictions is the observation of Snyder et al. (100) that a variety of protein modifying reagents and proteolytic enzymes in low concentrations do selectively reduce opiate agonist binding, with negligible effects on antagonist binding (106, 107).

The fact that several different ways of influencing protein structure, differentiate agonist and antagonists argues that these agents affect a variety of sites relevant to the interconversion of the two conformations of the opiate receptor. If there were separate agonist and antagonist receptors, as has been suggested by pharmacological evidence (108), one would have to assume that the agonist receptor possesses several aminoacid residues that are critical for receptor

binding and none which are components of the antagonist receptor.

Jacobson (68) noted that a few compounds appear to contradict the data of Pert and Snyder (99) in that they are predicted to be antagonists by their sodium:non-sodium ratio, but in vivo studies with monkeys show that they are not. Birdsall (275) has pointed out further discrepancies with the sodium response ratio. Although in their studies the binding properties of certain endorphins resembled those of the alkaloid antagonists, the endorphin (C-fragment) in question is a potent central analgesic (381) and acts as a full agonist on the guinea pig ileum (111). An alternative explanation for regulation of opiate ligand binding offered by Cardenas and Ross (276) views the opiate receptor in a Ca^{++} associated conformation. The binding of morphine induces Ca^{++} displacement shifting the membrane to a Ca^{++} -dissociated state which may be reversed by naloxone.

Endogenous Opiate Ligands

The receptor for a foreign drug is really the receptor for a humoral substance, with which the foreign molecule also interacts H.O.J. Collier (277).

A question posed, once the existence of specific opiate receptors had been demonstrated, was "Why are there opiate receptors?" Clearly, opiates are not normally consumed by most animals and clearly also, therefore, the opiate receptor must ordinarily interact with substances other than opiates, as Collier (277) had pointed out. More important the intrinsic activity of naloxone in various systems further supports the postulation of the existence of endogenous ligands. It has been observed that naloxone (a) increases acetylcholine release in the in vitro preparation of guinea pig ileum (278); (b) enhances the

nociceptive response in rats and mice (279); and (c) reverses electroanalgesia in rats (280). This led many investigators over the past few years, to initiate a search for the endogenous opiates.

In 1975, reports from the laboratories of Hughes (111) and of Terenius and Wahlström (79) claimed that opiate-like peptides could be identified in extracts from porcine brain (111) and from human cerebrospinal fluid (79). This was confirmed in other laboratories for many vertebrate species (282, 283, 284), and provided a possible explanation for the presence of the stereospecific opiate receptors. Since then numerous investigators using bioassays in mouse vas deferens, guinea pig ileum, stereospecific binding to purified brain extracts, and radioimmunoassays have reported the isolation of opiate-like peptides from blood, brain, pituitary and the guinea pig ileum (111, 272, 290, 291, 300). These endogenous opiate like ligands have collectively been named endorphins (292). The term 'endorphin' refers to any opioid-like substance (274). Natural endorphins (also called morphine-like factors) have now been extracted from all mammalian species studied, including pig, cow, rat, mouse, guinea pig, rabbit and man (293, 294, 295).

In December 1975, Hughes & coworkers (111) identified two pentapeptides (endorphins) from porcine brain that exhibited morphinomimetic activity. These pentapeptides have been called methionine-enkephalin (H-Tyr-Gly-Gly-Phe-Met-OH) and leucine-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) respectively. In this report (111), Hughes and his collaborators also called attention to the remarkable fact that the amino acid sequence of Met⁵-enkephalin was identical to that of the fragment Tyr⁶¹ to Met⁶⁵ of β -lipotropin. The latter is a minor component of

pituitary extracts for which no function has been determined since its isolation and characterization in 1964 by Li (296). The molecular structure of α , β (c-fragment), δ and γ endorphins, all isolated and characterized from the hypothalamus-neurohypophyseal tract, have respectively the same amino acid sequence as the fragments of β -lipotropin corresponding to residues 61-76, 61-91, 61-77, 61-87. It has been proposed that β -lipotropin, with no opiate-like activity of its own, would be the pro-hormone for all the endorphins and Met⁵-enkephalin (297). So far no precursor for Leu⁵-enkephalin has been either discovered or proposed. Thus, a large number of different degradation products of β -lipotropin have opiate activity. Furthermore, the stability and therefore the duration of action of these peptides varies widely, from the very short half-life of the enkephalins to the very long half-life of the complete 61-91 fragment (302). Day *et al.* (299) have proposed that the 'active' endogenous peptides all contain the N-terminal sequence H-Tyr-Gly-Gly-Phe OH. It has also been shown that enkephalins or peptides containing this structure are in fact natural levo-isomers, contain the basic nitrogen function and aromatic ring structures and thus possess all specific features of the opiate agonists (303). Klee (302) has suggested that when enkephalin binds to the receptor, it assumes a morphine-like conformation with the tyrosine moiety corresponding to the tyramine portion of the morphine molecule and the side chains of residue 5 and perhaps 4 as well interacting by hydrophobic forces with portions of the receptor that also interact with the C and D rings of opiates. It would appear that opiate receptors are in fact peptide (i.e. endorphin) receptors and that because of structural similarities, opiates only mimic the effect of these natural endogenous ligands.

To date the enkephalins have not been characterized in extracts of the pituitary gland, although the larger endorphins have been shown to be present in the brain (297, 298). Other morphinomimetic compounds have been isolated from the blood of various animals viz: anodynin (300) and fast moving material (FMM) and slow moving material (SMM) (290). Anodynin appears to derive from the pituitary gland, because it vanishes from the blood of hypophysectomized rats (274). An endogenous antagonist 'antendorphin' has been shown to be present in extracts of rat brain (301).

The endorphins like the opiates have been shown to have numerous actions in various test systems.

In synaptosomal opiate-binding assays, on application to single neurons by microiontophoresis (297), in analgesia studies (304, 305), in the isolated guinea-pig ileum, in the mouse vas deferens (306), as a stimulus for growth hormone release (307), in inhibiting adenylate cyclase in neuroblastoma x glioma cell homogenates (308), in producing euphoria (304), in producing physical dependence (297, 309, 310), and in many other experiments, the pharmacological profile of the endorphins has been shown to resemble that of the opiate agonists.

It is believed that some or all of the endogenous ligands may have a neuromodulator or neurotransmitter role and the following is the information available regarding the status of enkephalins as putative neurotransmitters (295):

- (a) The pentapeptides are present in brain (16-21).
furthermore, they are present in specific areas of brain in association with stereospecific receptors (311, 312, 313, 314) and are apparently localized in

nerve terminals (311, 315, 316).

- (b) The enzymatic machinery has not been identified but synthesis from labelled precursor has been observed in brain (317).
- (c) While specific synaptically located enzymes have not yet been identified, a highly effective system for inactivation of enkephalin exists in brain (318, 319, 320, 321, 322, 323).
- (d) Evidence, mostly indirect has been obtained for release both from isolated guinea pig ileum (324, 325, 326) and from brain in vitro and in vivo (79, 279, 280, 311, 315, 327, 328, 329, 330, 331, 332, 333).
- (e) The enkephalins have a predominantly depressant action, apparently at a postsynaptic site, on single neurons in the particular brain areas where they and opiate receptors occur and this can be antagonized by naloxone (334-341).

It seems both unnecessary and unwise to argue from such facts that one or the other type of peptide represents the physiologically significant principle. Both short-acting peptides, serving as classic neurotransmitters, and longer-acting ones, serving as modulators of neural pathways, can be physiologically important (302).

If the endorphins are neurotransmitters, how might they affect postsynaptic cells? Morphine and other opiates applied iontophoretically to single cerebral cortex cells, inhibit firing in proportion to their pharmacological potency with marked stereospecific actions and antagonism by naloxone, suggesting that the morphinelike factor may

be an inhibitory transmitter (22).

There are three possibilities of control of an inhibitory mechanism by enkephalin (Figure 2).

The inhibition could be postsynaptic, presynaptic or could be due to a modulatory effect on the nerve terminal in which both enkephalin and the compound responsible for interneuronal transmission are present. In the first two circumstances, two neurones would be involved, while in the third circumstance, enkephalin would be released from the nerve terminal on which it acts by inhibiting the release of the neurotransmitter.

Chemical Composition, Location, and Distribution of Opiate Receptors

In plexus-free strips of intestinal longitudinal muscle of guinea pig ileum, all opiate receptor binding was abolished indicating that the opiate receptor is confined to nervous tissue (118).

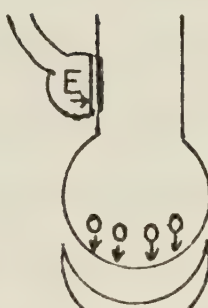
The opiate receptor distribution has been determined within neurons, throughout the various regions of the vertebrate brain and across phyla. The isolated receptors are highly enriched in synaptic membranes of some class of neurons which are heterogeneously scattered through most regions of the primate CNS, particularly in the limbic system. Since all of the putative neurotransmitters fail to inhibit opiate receptor binding and no good correlation with any known neurotransmitter specific system in the brain is apparent, it is unlikely that the opiate receptor is closely and exclusively associated with one of the known neurotransmitter systems (85, 121, 226).

Lesioning experiments (85, 119, 120, 121) indicate that the opiate receptor is not a unique component of axons or nerve endings of any one of the known neurotransmitter tracts.

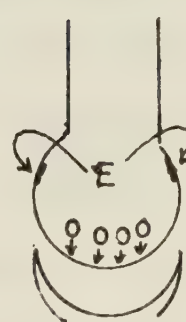
Postsynaptic
Inhibition



Presynaptic
Inhibition



Inhibitory
Modulation



E = Enkephalin

Figure 2: Possible mechanisms for the action of enkephalin (adapted from, 233).

The apparent absence of "opiate receptors" in brains of invertebrates, suggests that these drugs do not elicit specific pharmacological responses in invertebrates (109).

Opiates may exert their actions primarily in the synaptic region between neurons. However, such a conclusion must be drawn with caution, because fractions obtained in these subcellular fractionation procedures are rarely "pure". Moreover, in synaptic membrane fractions, one cannot discriminate between presynaptic and postsynaptic membranes, therefore it is not possible to determine by these techniques at which side of the synapse opiates act. Using recently identified receptor binding for several neurotransmitters in the brain as postsynaptic markers, some investigators (123, 124) have attempted to develop techniques for separating presynaptic and postsynaptic membranes and by using these techniques to localize the opiate receptor with greater precision.

Lowney et al. (127) described the solubilization and purification of a lipid-rich brain substance that binds levorphanol stereospecifically and saturably. Loh et al. (128), showed that this binding could be accounted for by cerebroside, which may or may not be related to the opiate receptor. Loh (129) also emphasized the complementary structure of cerebroside to opiates. If cerebroside sulfate binds morphine, the question arises concerning the relationship of this effect to the pharmacological actions of opiates. Jimmy mice have a low brain content of cerebroside sulfate (129). In these mice the cerebroside sulfate concentration in the brain is only 60% of control-mice. These mice are very resistant to the pharmacological actions of morphine, requiring six times more morphine to produce analgesia than

normal mice. This insensitivity to morphine cannot be accounted for by penetration of morphine into the brain. Because cerebroside sulfate does bind opiates with high affinity and the binding shows some correlation with pharmacological potency, it may be related in some way to the opiate receptors (129). Cerebrosides might be prosthetic groups of the opiate receptor, as heme is related to globin in hemoglobin function (130).

Pert (132), pointed out that there is a positive correlation between the effective analgesic sites in the brain and those areas found to be rich in opiate receptor binding sites by Kuhar and co-workers (121) and by Hiller et al. (120). Thus, the regions of the medial thalamus, periaqueductal and periventricular gray matter and hypothalamus that are rich in opiate receptor binding sites appear to be most effective in producing analgesia when injected with morphine. The major discrepancy appears to be in the amygdala, an area rich in opiate binding in which morphine was found to be ineffective, in producing analgesia. Nauta (131) also suggested that opiate receptors in the amygdala might not be related to the analgesic properties, but to other pharmacological actions of morphine.

A number of investigators have speculated that the development of tolerance and physical dependence are mediated by changes in opiate receptors (134, 135, 136, 137), i.e. these theories have been based upon postulated changes in the number of opiate receptors. However, Pert et al. (138) could detect no change in the number of isolated opiate receptors which could be construed as relevant to the development of tolerance or physical dependence. They proposed an alternative hypothesis, which is that a gradual shift in the equilibrium between

the two postulated conformations of the opiate receptor occurs, which would progressively favor the "antagonist" conformation over the "agonist" conformation. This postulated change in the receptor equilibrium to favor the antagonist conformation could occur by a gradual migration of receptors through membranes to regions having a higher sodium concentration, perhaps from inside to outside of the cell, or merely by a re-organization of receptor protomers within the membrane which would alter the cooperativity to favor the antagonist conformation.

Perhaps, the development of an irreversible label for the receptor would shed light on this problem of addiction and physical dependence. One would then be able to measure the rate of turnover of the opiate receptor molecule itself. Perhaps alterations in the rate of receptor turnover, underlie the process of addiction, although it is quite conceivable that long term changes of some other, as yet obscure nature are involved.

The actions of sodium on opiate binding described by Snyder's group (100) suggest that these receptors may be located on the external surfaces of cells where they would normally be exposed to relatively high sodium ion concentrations. Hitzemann & Loh (140) showed that their stereospecific narcotic binding material is probably located on the external surface of the nerve ending particles ("synaptosomes"). They showed that after tryptic digestion of nerve ending particles, the stereospecific narcotic binding is eliminated. This is keeping in line with the fact that trypsin does not penetrate through the membrane and thus only those proteins which are partially or completely located on the external surface of the membrane

will be susceptible to tryptic activity (222).

Opiate Receptors and Sodium Conductance

Several drugs and toxins are known to interfere with sodium movements through biological membranes and the opiate receptor could be involved in the control of these sodium movements.

Tetrodotoxin had no effect on naltrexone binding and TTX did not block the sodium enhancement of naltrexone binding (141). These findings indicate that the opiate receptor does not have any TTX binding site.

Some local anesthetics carry a cationic charge and act at a "receptor site" on the axoplasmic end of the sodium channel (142). In addition, non-ionic local anesthetics, e.g. benzocaine, are thought to block nerve conduction by disorganizing the membrane structure around the sodium channels and in this way block sodium conductance (67).

The existence of certain similarities between the opiate and the local anesthetic receptor is suggested by the following findings (141):

- (a) there is a direct competition between local anesthetics and opioids for binding to the isolated "opiate receptors".
- (b) this competition takes place with concentrations of local anesthetics that produce nerve block.
- (c) the more potent local anesthetics are also more potent inhibitors of opiate stereospecific binding, and
- (d) other drugs such as antihistamines, antiarrhythmic drugs, tranquilizers, etc., that have local anesthetic effects in high concentrations also interfere with stereospecific opiate binding.

The finding that cationic local anesthetics act competitively with opioids suggests that the cationic group of local anesthetics interacts with a specific anionic binding site on the opiate receptor. That the inhibition of naltrexone binding by benzocaine is non-competitive is consistent with the disrupting effect that non-cationic local anesthetics are thought to have on membranes (141, 228).

Despite certain similarities between the opiate and the local anesthetic "receptor", it is evident that they are two distinct structures. Regional distribution studies indicate that the opiate receptor is not uniformly distributed (120, 121), in nervous tissue, while all nerves are susceptible to the effects of local anesthetics. The cellular localization of both "receptors" also seems to be different. The local anesthetic "receptor" is intracellular (142) while the opiate receptor should be extracellular in order to be accessible to the endogenous ligand, which is known to be a polypeptide of relatively large size (143, 144) and therefore not expected to pass through cell membranes unless there is a specialized uptake system.

Receptor-Composition

Despite numerous experiments (108, 164, 342, 343, 344) the exact composition of the receptor unit(s) and the nature of the interaction of opiate drugs and receptors remains elusive. The problem that remains unresolved is whether these drugs act and interact on a single 'type of opiate drug receptor', interact by altering the conformational characteristics of the receptor molecules at this single site, or act on and interact by uniting with two or more different but closely associated receptor sites.

Initially it was believed that narcotic analgesics and antagonists

acted competitively at the same receptor site because of the structural similarities between active compounds (69, 145, 146, 147) and because of pharmacological observations (148, 149, 150, 151).

Wikler (152), emphasized that the high degree of pharmacological specificity which characterized the antagonism of opiates must be a consequence of molecular competition between opiate antagonists and agonists at cellular receptor sites. Thus, it was speculated that opiate antagonists must act at the same receptor sites of morphine action. Moreover, since opiate agonists and antagonists are so structurally similar, it was difficult to envision how they could have different receptor sites (151, 153). Consistent with this idea, evidence accrued that antagonists do not prevent the drugs from entering the brain of the intact animal (154, 155).

Initial observations with drugs, e.g. nalorphine led to the conclusion that these drugs were partial agonists and interacted with full agonists at the same receptor site according to the principles of "Competitive Dualism in Action" as described by Ariens et al. (156).

In 1967, Martin concluded that 'Competitive Dualism in Action' at a single receptor site could not account for the results obtained in several agonist-antagonist drug interaction studies. Thus, Martin proposed a theory of 'Receptor-Dualism', which stated that there are in fact two types of opiate drug receptors; at one type full agonists act to produce an effect and partial agonists act to produce only antagonism and another type at which only partial agonists act to produce an effect. A "pure" antagonist, e.g. naloxone has antagonistic effects at both sites.

From their experiments Takemori et al. (157, 158) concluded that

the narcotic and narcotic antagonist analgesics interact either with two different receptor populations or with the same receptor in a different manner. Thus an extension of the suggestion of Portuguese (69, 160) was formulated in which different narcotic analgesics are viewed as binding with a single species of receptors but having different positions of molecular binding (Figure 3). The protonated nitrogen (N^+) is assumed to be the common site of attachment to the receptor. Both the full agonists and partial agonists unite with site "N" to produce an apparent competitive antagonism at site "N". Chronic interaction at the "N" site desensitizes the site resulting in tolerance and simultaneously increasing the binding constants of the antagonists for site "NA" resulting in an increased sensitivity to antagonism (161, 162, 163).

The observations of Smith et al. (345), suggested that the analgesic, respiratory depression, cataractogenic and growth-inhibiting responses to opiate drugs are each mediated by receptors differing on the basis of the degree of tolerance development in each of the four parameters. Gullemin (297) showed that each of the endorphins, has unmistakable effects of its own, in addition to some common properties between the different endorphins. Thus the possibility has been raised that the various endorphins are more or less specific to particular opiate receptors (298). This in turn, would imply a heterogeneity of opiate receptors that has been proposed (346) on the basis of pharmacological data. Martin (346) proposed three types of receptor, designated μ , κ and σ , based upon differential effects of various classes of opiate agonists and mixed agonist-antagonists. Lord et al. (348) proposed another class of receptors (δ -receptors)

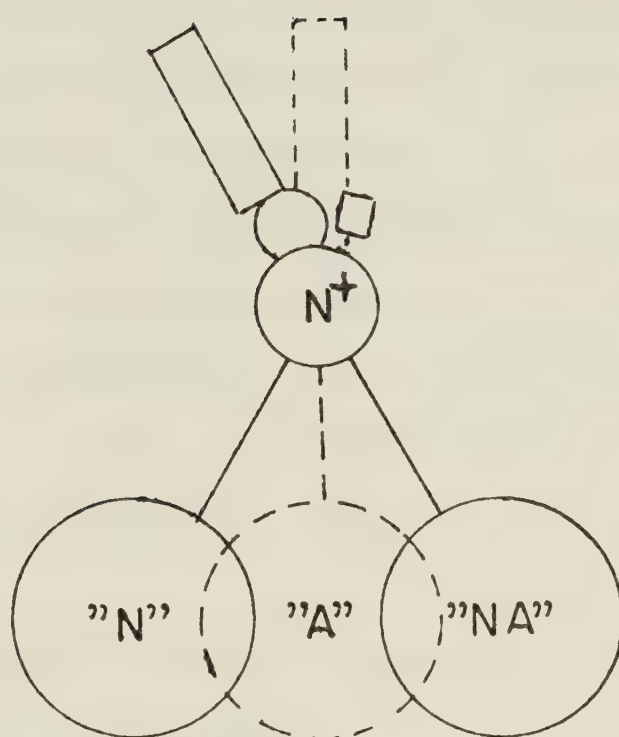


Figure 3: Hypothetical interactions between narcotic analgesics or antagonists and the analgesic receptor. N=narcotic analgesic site, A=antagonist site, NA=narcotic antagonist analgesic site (adapted from, 164).

for the mouse vas deferens. Hutchinson et al. (347) showed that the receptors in guinea pig myenteric plexus and in the mouse vas deferens behaved differently with respect to relative affinities for agonists and antagonists. Direct binding studies with [^3H] leu-enkephalin also indicate the existence of multiple receptor sites in brain (348).

These observations on the heterogeneity of the opiate receptors are strongly reinforced by the behaviour of the endorphins. No correlation (i.e. the rank order potency did not vary in parallel) between two in vitro assay models (mouse vas deferens and the guinea pig ileum) was found for the enkephalins by Lord et al. (348).

To add to the above confusion Puig and co-workers (349), showed that the reversal by opiate antagonists of the inhibition by opiate agonists of the electrically evoked contractions of the guinea pig ileum, was independent of the general structure of the antagonists, implying that a heterogeneous group of opiate receptors, did not exist.

Furthermore, the evidence available at present is insufficient to attempt to allocate different receptors to different physiological functions.

Problems with Receptor Isolation Studies

Advances in isolating membrane bound receptors have been relatively slow. First, the well developed methods of fractionating and purifying water-soluble proteins are often inapplicable. Second, drugs that act in the CNS, since they have to penetrate the Blood Brain Barrier (BBB), are necessarily lipophilic. This complicates receptor binding studies, because the membrane receptors are imbedded in a

lipid matrix, with which such drugs will interact through nonspecific hydrophobic forces. Thus, the non-specific binding of the opiates obscures the specific binding which is believed to be responsible for the actual narcotic effect. Third, the reversibility of most drug-receptor binding implies that significant binding sites may be overlooked because of dissociation of drug molecules from the receptors during work-up procedures. Goldstein et al. (165) pointed out that the opiates contain a protonated nitrogen atom at physiologic pH, suggesting the likelihood of non-specific interactions with numerous anionic groups in tissues; and that they are also lipophilic, passing readily through the BBB and presumably associating nonspecifically with neuronal membranes and myelin. They suggested, however, that stereospecificity might provide the key to receptor isolation in this drug family.

However, stereospecific binding, while necessary, is not a sufficient criterion for the opiate receptor. Pasternak & Snyder (166) demonstrated that filters themselves were capable of binding opiates in a stereospecific fashion. Since glass filters can bind opiates stereospecifically, certain macromolecular brain or other constituents probably also exhibit stereospecific opiate binding which, like the glass filters is not related to the pharmacological actions of opiates. Various lipids also displace stereospecific binding of opiates with the levo-isomer more active than the dextro-isomer (128, 350). Furthermore often than not, the methods employed by most "binding" investigators lend themselves to the study of only high affinity sites, because the tissue fragments are washed before radioactive counting, thus removing much or all of the more loosely bound drug. To what extent

the drug that dissociates and is lost was also stereospecifically bound is unknown.

The main reason however that receptors have proved difficult to characterize and isolate lies to a large degree in the absence of criteria for demonstrating that one has a receptor after it has been removed from its normal functional connections in vivo.

One must be cautious about equating tissue binding with receptor function even if the binding examined is saturable at low concentrations. Hollenberg and Cuatrecasas (167) observed that insulin binding to glass saturates at extremely low concentrations, similar to the affinity of the true insulin receptor for insulin. Additional problems with in vivo studies of drug-receptor interactions are the effects of distribution and metabolism on the proportion of the administered drug that actually reaches the receptor site, the effects of compensatory physiological and biochemical processes on the responses recorded, and the possibility that the response recorded is so far removed from the site of the drug-receptor interaction that the response is limited or otherwise modified by intervening physiological or biochemical processes (e.g. if a response is dependent upon the release of a neurotransmitter, it will be limited by the amount of transmitter available for release).

Further, the number of types of opiate receptors has not been established. Is there only one kind of opiate receptor or are there different receptors mediating the various actions of opiates? Do the receptors exist in several conformational states and do they have several different binding sites? Finally, it still remains to be demonstrated that the binding of narcotics to the putative opiate

receptor material initiates a pharmacological response.

Some investigators have attempted to isolate material which will specifically bind opiates but not inactive stereoisomers (66, 80, 82, 98, 99, 165, 170, 171). It is concluded from such studies that this isolated specific binding material is in fact the opiate drug receptor. Attempts are then made to relate the physiological effects of opiate drugs and the interaction of these drugs to their binding properties with this material. Inherent in such studies is the assumption that receptor binding is sufficient to explain drug response and that drug properties such as efficacy or intrinsic activity are of no importance and that spare receptors, either do not exist or have properties identical to the true receptors, but in any event do not modify the interpretation of the results obtained in binding studies (118, 173). However, there seems to be little valid justification for these assumptions. Another problem with these studies is that different investigators often differ as to the cellular fraction in which the material is found and its amount.

Although it is clear that there are binding sites with intriguing specificity, there is not yet proof that the binding site-opiate complex is biologically active. Stereospecificity, may not be a sufficient condition for biological activity, as pointed out above. The high affinity of the binding site is also not proof of functional activity; the binding constant should be appropriate to the biological concentration in the neighbourhood of the in vivo receptor.

In Vivo Studies of Opiate Receptors on Excitable Membranes in the CNS

Many such experiments are conducted under acute surgical conditions with anesthetized animals. The use of anesthetic agents can be expected

to alter the evoked response to noxious input and interact with morphine action by either potentiating its effects or masking them.

Several microiontophoretic studies employing opiates and endorphins have been conducted in the CNS. Microinjection studies provide one of the critical tests of the identity of a substance as a transmitter as the putative transmitter is placed close to the cells presumed to be excited or inhibited by it, while the firing activity of the cells is monitored continuously.

The effects of microiontophoretically applied morphine and levorphanol on the spontaneous firing of neurons in the locus coeruleus of rats were investigated, by Korf et al. (177) and Bird & Kuhar (351). Both groups of investigators reported that opiate agonists depressed spontaneous activity, and this depression could be antagonized by naloxone and levallorphan. Studies in other areas of the CNS namely the cerebral cortex, thalamus, dorsal medulla (337, 352), caudate nucleus, periaqueductal grey matter (340, 352), substantia gelatinosa (353), brainstem neurones (334, 335, 336, 352), spinal neurones (338, 339), nucleus accumbens (354), trigeminal nucleus caudalis (355), with either opiate agonists, or endorphins or both have shown that by and large these agents cause a depression of spontaneous, or evoked activity. In most cases this depression could be prevented or antagonized by opiate antagonists. In very few cases when application of opiate agonists or endorphins caused excitation, this excitation was either not naloxone antagonizable or not stereospecific. A striking feature of the above experiments is that this inhibitory effect of opiates and endogenous ligands occurs whether the neurones are firing spontaneously (brain stem: 334, 335); firing

spontaneously or firing in response to noxious stimuli (locus coeruleus: 177, 351); firing spontaneously or excited by glutamate or acetylcholine (cortex: 201); or firing in response to aspartate, or glutamate (spinal sensory interneurons: 356). It seems likely that the effect of morphine at these sites is not related to an antagonism of any specific excitatory stimulus, but rather represents an opiate receptor mediated membrane stabilization.

Some investigators have reported (341, 352, 357) excitation of either Renshaw cells or the hippocampus on application of morphine, endorphins or substance P. These investigations showed that in contrast to other areas of the CNS, such excitation could be prevented or antagonized by application of opiate antagonists.

These microiontophoretic studies suggest that opiates and endorphins may exert at least part of their activity through alteration of impulse flow in certain areas of the CNS.

In Vitro Isolated Tissue Studies of Opiate Receptors

Efforts have been made to conduct opiate drug receptor studies on an isolated in vitro preparation. One of the problems encountered are the findings that the same tissue or preparation from different species differed qualitatively in their sensitivity to opiate agonists (182).

In most of such studies, narcotic antagonists when employed are used mainly to distinguish between a non-specific or an opiate drug receptor mediated effect. In some preparations, opiates inhibit the release of neurotransmitters from presynaptic nerve endings. This has been shown for the guinea pig ileum (84, 94, 183), the superior cervical ganglion of the cat (184, 185), the nictitating membrane of the cat (184, 186), the neuromuscular junction of the frog (187), etc.

The most comprehensive investigation of narcotic drug actions and interactions on an isolated preparation has been conducted by Kosterlitz and his co-workers, on the inhibition of the response to coaxial stimulation of the guinea pig ileum (182, 188).

The results they obtained using this preparation were well correlated with the analgesic effects of these drugs and showed also that this preparation was useful for drug-interaction studies. Although this preparation has obvious advantages over in vivo studies, it still has the problem that the response recorded is only secondarily related to the physiological changes produced by the action of the opiate drug on its receptor.

Some investigators prefer to think that CNS drugs produce depression by interfering with synaptic transmission processes, even though this would mean that a variety of mechanisms are involved to correspond to the variety of effects produced by these drugs on synaptic transmission processes (190, 360, 361).

With several peripheral preparations, notably the guinea pig ileum, the mouse vas deferens and the frog neuromuscular junction, it has been shown that opiates disrupt synaptic transmission by suppressing the neurotransmitter release. However, despite the general consensus that opiates inhibit neurotransmitter release, there is considerable controversy regarding the mechanisms underlying the suppression of neurotransmitter release by these drugs.

Schaumann (183) and Paton (84) demonstrated that morphine and its surrogates are powerfully effective in blocking the contraction of electrically stimulated ileum strips. Paton (84) showed, further, that this effect is closely linked to the reduction of acetylcholine release

into the bath following each stimulus. It has been shown by others (111, 290, 362) that endogenous ligands are not only present in the guinea pig ileum but also like the opiates inhibit acetylcholine release.

Dingledine and Goldstein (200) suggested that opiates probably act to block impulse invasion into the terminal varicosities of the cholinergic neuron. Intracellular studies by North and colleagues (363-367) showed that neurones in the isolated myenteric plexus of the guinea pig ileum responded with membrane hyperpolarization on exposure to opiates and enkephalins. This hyperpolarization was prevented by naloxone. The hyperpolarization was usually associated with a fall in membrane resistance. These effects they concluded were sufficient to prevent excitation of a population of myenteric neurones. This they suggested would reduce the output of acetylcholine when the preparation was field stimulated. These findings lend some support to the hypothesis that the central inhibitory actions of opiates and endogenous ligands may be mediated by a membrane hyperpolarization associated with a conductance increase.

Henderson and Hughes (368) quoted reports stating that the motor innervation of the mouse vas deferens is adrenergic. They also demonstrated that opiates and enkephalins inhibit the release of nor-adrenaline from the motor nerve terminals and thus prevent electrically induced contractions of the vas deferens. These inhibitory effects could be antagonized by naloxone.

Henderson and North (369) made intracellular recordings from single smooth muscle cells of the mouse vas deferens. Opiates and

enkephalin depressed the amplitude of the excitatory junction potentials. These drugs did not affect the resting membrane potential of the smooth muscle cells. Their action was antagonized by naloxone. These workers concluded that opiates and enkephalins act directly upon the transmitter release sites, to reduce the amount of noradrenaline liberated by nerve impulses.

Frederickson et al. (370) and Frederickson and Pinsky (371) found that acetylcholine release in the rat phrenic nerve-diaphragm and frog sartorius preparations was reduced by morphine. Bell and Rees (372) examined this question using a rat diaphragm preparation and found no antagonism by naloxone of the depressant effects of agonists on neuromuscular transmission. They concluded that these effects on the neuromuscular junction were not due to a receptor mediated process.

From all the above studies on the effects of opiates and endogenous ligands on synaptic transmission the following conclusions can be drawn: (a) It is quite probable that opiates have synaptic actions, and it is also probable that the identity of the transmitters contained at the synapse is not a critical factor in determining whether a given synapse is or is not affected by these drugs; (b) It is likely that interference with regulatory events at the subsynaptic level, such as those controlling second messengers, could account for opiate effects just as effectively as interference with presynaptic events such as transmitter release and reuptake. The potential interactions between the drugs and divalent cations (especially Ca^{++}) binding sites may be extremely important, since synaptic events are strongly dependent on the availability of these elements.

There is considerable evidence to show that drugs with general CNS depressant properties also depress excitability and action potential production in isolated cells (67), and when tested they are found to act by depressing the specific increase in Na^+ conductivity which normally results from an adequate stimulus (189, 190).

Hasbrouck (65) reported that naloxone, a narcotic analgesic antagonist could antagonize the general CNS depression produced by morphine in man. This was a surprising observation, because among other possibilities it would suggest that there are opiate drug receptors on excitable cells.

Ritchie and Armett (358) reported that high concentrations of morphine and nalorphine applied topically antagonized the depolarization of vagal C fibres by acetylcholine, but without blocking action potential conduction by themselves. Kosterlitz and Wallis (191) found that morphine had no effect on conduction by mammalian peripheral nerve. Simon and Rosenberg (193) used invertebrate (squid and crustacean) axons. Relatively high concentrations of morphine and levorphanol decreased spike amplitude and even blocked conduction. These drugs also reduced or blocked repetitive firing in squid axons in media with low divalent cation content. Agonists (morphine, levorphanol), antagonist (levallorphan) and the inactive isomer of levorphanol (dextrorphan) had similar effects, suggesting to them that local anesthesia by membrane stabilization was responsible for the block. Frazier and his colleagues (194, 359) injected morphine inside previously evacuated squid axons and found that it blocked axonal conduction not by changing the resting membrane potential but by depressing transient ionic (Na^+ and K^+) conductance changes, very much like

local anesthetics and the aliphatic alcohols. In their second paper (359), Frazier et al. showed that two antagonists naloxone and M5050, as well as etorphine, a potent agonist had qualitatively and quantitatively the same effects as morphine. Frazier et al. (194), therefore concluded that the depressant effects of narcotic analgesics on excitability were not due to an action on an opiate drug receptor having properties comparable to the opiate receptors found in the CNS or in smooth muscle. Seeman et al. (192) reported that the minimal blocking concentrations on rat phrenic nerve did not differ for D- and L-methadone and was only slightly higher for dextrorphan than for levorphanol. These results were assumed to show that stereospecific binding sites for opiate drugs do not exist on excitable membranes.

Frank (196) studied the effects of morphine, meperidine and naloxone applied externally to frog's sartorius muscles. Thus when applied at a concentration of 10^{-3} M both naloxone and morphine reduced the maximum amplitude of the extracellularly recorded compound action potential by about the same amount (30%).

In contrast to the agonist effects of high naloxone concentrations, it was found in this study (196) that low naloxone concentrations (e.g. 3×10^{-7} M) antagonized the depressant effects of morphine or meperidine on action potential production in frog's sartorius muscles. These results demonstrated that there were opiate drug receptors on sartorius muscle fibre membranes and that drug activation of these receptors inhibits action potential production.

In the above study, there was never any sign that at low, antagonistic doses, naloxone could produce effects opposite to those of morphine or meperidine. Therefore, a physiological type of antagonism

could be ruled out. Neither did naloxone at any dose antagonize the effects of phenobarbital on intact mice. Thus, there was no indication of any non-competitive antagonism. These results indicated that naloxone is a partial agonist (or antagonist) with a very low intrinsic activity (196). Results supporting the contention that opiate antagonists do not induce a physiological type of antagonism to the depressant effects of opiates on excitable membranes have been obtained by Jhamandas (374) and Frank and Buttar (373).

In a subsequent study with intracellular microelectrodes (56) it was shown that meperidine blocked action potential production by two mechanisms: (a) a non-specific mechanism in which the increase in sodium conductance (\bar{g}_{Na}) and in potassium conductance (\bar{g}_K) produced by stimulation are depressed and (b) an opiate drug receptor mediated mechanism, causing a specific depression of \bar{g}_{Na} . Low, antagonistic concentrations of naloxone could antagonize only the effects produced by the second mechanism (b). These results also indicated that there are opiate drug receptors located probably on the inner surface of the muscle membrane associated with the "sodium channels" and that drug activation of these receptors by either meperidine or high naloxone concentrations interferes with the opening of the "sodium channels" normally produced by membrane depolarization.

The inability of Kosterlitz and Wallis (191) to demonstrate an inhibitory effect of morphine and some related drugs on action potential production in mammalian nerves was probably due to their use of low opiate concentrations.

For studying the effects on nerves in situ in the cat, Kosterlitz and Wallis (191) used a 3mg/kg dose of morphine equivalent to the 1-6

mg/kg dose of morphine sulfate (M.Wt. 758.8) required to produce general anesthesia in man (64, 65). Since the function of peripheral nerves is generally unaffected by drug concentrations which produce general anesthesia (190), the lack of effect on peripheral nerves reported by Kosterlitz and Wallis (191) in their *in situ* studies is not surprising. Thus, despite the potential error always inherent in comparing drug dosages in different species, it seems reasonable to conclude that they failed to demonstrate the depression of action potential production by narcotic drugs, because the drug doses they employed were too low (195). It is also probable that under the conditions of their experiments, Seeman et al. (67) and Frazier et al. (194) observed only the non-specific blocking effects of opiates. The possibility that opiates may alter membrane excitability by affecting membrane permeability has been supported in studies conducted by Zieglgansberger and colleagues (201, 338, 339, 376, 377). These workers initially showed that the depolarizing action of L-Glutamate upon spinal neurones was associated with a marked increase in postsynaptic permeability for Na^+ ions. They subsequently demonstrated that opiates block L-Glutamate-induced depolarizations by impairing the Na^+ influx and by slowing the rate of rise of excitatory postsynaptic potentials (EPSP's) in a naloxone-antagonizable fashion. Further, higher doses of opiate agonists (e.g. morphine) and antagonists (e.g. levallorphan) and inactive stereoisomers (e.g. dextrorphan) produced a non-specific local anesthetic-like action. Where morphine was applied intracellularly while glutamate was applied extracellularly, the blocking action of morphine was no longer demonstrable indicating that opiate receptors in this area of the brain were located extracellularly.

Haloperidol has been shown to antagonize withdrawal symptoms in morphine dependent rats and to compare favorably with methadone in blocking heroin withdrawal symptoms in man (198). Haloperidol is a chemical derivative of meperidine (199) and it is possible that its effects in this regard, are due to an action on opiate drug receptors. Findings in our laboratory indicate that haloperidol has an agonistic effect on opiate drug receptors on excitable membranes and part of this effect like that of meperidine can be antagonized by employing concomitantly low concentrations of opiate antagonists (375).

Although a reduction in membrane permeability may account for the ability of narcotics to block excitability, the exact mechanism by which the permeability is affected remains speculative. One thing is certain that most depressant drugs depress \bar{g}_{Na} , but whether they reduce \bar{g}_{Na} by the same mechanism is uncertain (206). It may be that some of the effects of depressant drugs that reduce \bar{g}_{Na} are not mediated via the classical pharmacological receptors, because a chemically heterogenous group of drugs appears to be having the same final effect.

In summary opiate receptors are hypothetical cellular constituents, which have been invoked as the most parsimonious explanation to account for an abundance of pharmacological phenomena, produced by the opiate drugs.

III. STATEMENT OF THE PROBLEM

The aim of this thesis was to carry out an electrophysiological analysis of the stabilizing action of different opiate drugs and local anesthetics on action potential production in frog's sartorius muscle in order to clarify the mechanism of action by which these drugs exert their effects. An important objective was to determine the nature, specificity and sensitivity of the opiate receptors on this muscle by studying the interaction effects of the above drugs. Another objective was to test the hypothesis that suppression of sodium conductance plays a role in the membrane action of narcotic drugs, and in fact, that this is the main mechanism by which such drugs depress membrane excitability.

IV. MATERIALS AND METHODS

A. Muscle Preparations

The sartorius muscle of the leopard frog (*Rana pipiens*), was used throughout this investigation. Each frog was killed by a blow on the head and the muscle dissected and removed with a portion of the pelvic girdle attached. After removal, the muscle was placed in a Lucite bath and with the aid of a dissection microscope (Wild - Heerbrigg, Switzerland) it was freed from connective tissue and fascial membranes. Occasionally the sartorius muscle from the frog *Rana temporaria* was used.

All experiments were performed at room temperature (21-22° C.). The muscles were usually allowed to equilibrate in Ringer's solution for about 60 minutes before any recordings were conducted.

B. Solutions

The Ringer's solution was prepared in distilled water. The composition was as follows (mM): NaCl - 111.8; KCl - 2.47; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ - 1.08 (1.80 for *Rana temporaria*); NaH_2PO_4 - 0.087; NaHCO_3 - 2.38; and dextrose - 11.1. This solution had a pH of about 7.4 → 7.6, and contained also d-tubocurarine (ICN Pharmaceuticals Inc., Cleveland, Ohio) in a concentration of 10^{-4} g/ml. Tubocurarine was employed to eliminate possible neuromuscular effects. Certain solutions were made up with excess or reduced sodium chloride, the latter by substituting sucrose to maintain osmolarity.

C. Drugs

Drugs employed in this study were:

(a) Morphine hydrochloride - May & Baker

(b) Methadone hydrochloride - May & Baker

- (c) Propoxyphene hydrochloride - Eli Lilly & Co.
- (d) Meperidine hydrochloride - Winthrop Labs.
- (e) Naloxone hydrochloride - Endo Labs Inc.
- (f) Naltrexone hydrochloride - Endo Labs Inc.
- (g) Dextromethorphan hydrobromide - Hoffman - La Roche Ltd.
- (h) Procaine hydrochloride - Matheson Coleman & Bell
- (i) Tetrodotoxin - Sankyo Co.

The desired drug concentrations were made with Ringer's solution. All concentrations refer to the final bath concentration and are expressed as μM or M. All solutions were millipore filtered through millipore filter ($0.22 \mu\text{M}$) before use.

D. Electrodes

Electrodes were made from open ended capillaries (Fisher Scientific Co.) having 1.5-2 mm outside diameter. The microelectrodes were drawn by a glass microelectrode puller (PN-3 Narishige Scientific Instrument Lab. Ltd., Japan). These microelectrodes were then mounted on a microscope slide with a rubber band around them and placed into a Coplin Staining dish with their tips downward. The electrodes were filled using the method described below. The Coplin dish was filled with methanol and placed in a vacuum dessicator. The methanol was allowed to boil for about five minutes by reducing the pressure in the dessicator. With this procedure, most of the electrodes were completely filled with methanol. The electrodes attached to this slide were then transferred to another Coplin dish containing distilled water and they were left there overnight. After 24 hours, the electrodes were placed in millipore filtered 3 MKCl and left there for at least 24 hours before use.

E. Extracellular Recording

Usually the isolated sartorius muscle was further dissected with the aid of the dissection microscope, until a bundle of fibres from the central portion (max. diameter ≤ 1 m.m.) remained. These muscle bundles were mounted horizontally on bipolar platinum stimulating and bipolar platinum recording electrodes in a Lucite box bath. A ground electrode touched the muscle in between (Figure 4).

In some cases, when very small sartorii muscles were used, the whole muscle was mounted for testing without dissection.

For each test of the electrical responses of the muscle strips, the bath was completely drained by suction and the interelectrode spaces carefully blotted with filter paper to eliminate short circuiting. The muscle was stimulated with 2 msec square wave pulses. First, the strength of stimulation was gradually increased to determine the smallest stimulus needed to produce a measurable action potential. This was considered to be the threshold. This threshold current was measured by recording the voltage drop across a 50 K resistor in series in the stimulating circuit, on one beam of a double beam oscilloscope (Textronix 502). Next, records were made of the maximum compound action potential produced by supermaximal stimuli and this bipolar extracellularly monitored action potential was recorded on the other beam of the oscilloscope. This entire procedure took about 3 minutes and then the muscle bundle was reimmersed in solution. The amplitude of the initial negative spike of the bipolar action potential was used as the measure of the maximum amplitude of the compound action potential. The horizontal time between the end of the current pulse in the top beam and the initial peak of the

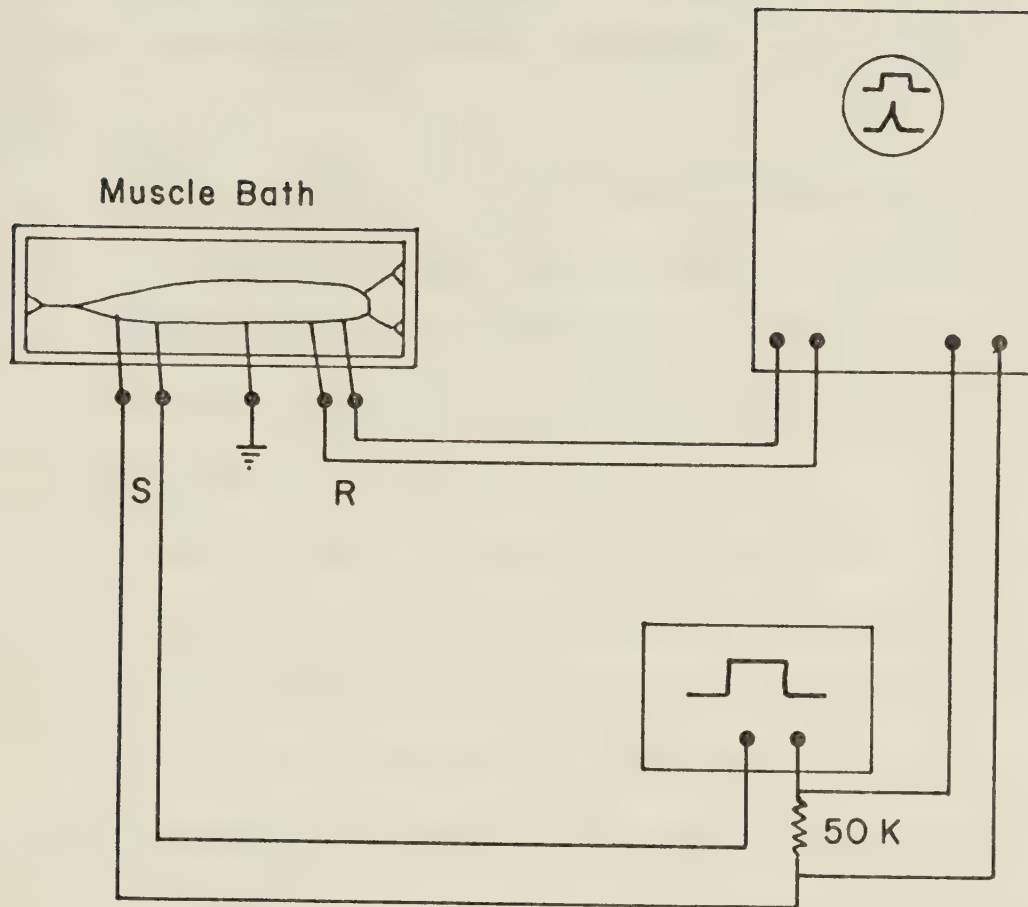


Figure 4: Block diagram of assembly used for extracellular studies. S, Stimulating electrodes; R, Recording electrodes.

bipolar action potential was used as a measure of the conduction velocity (after taking the distance between the recording and stimulating electrodes into account).

The excitability was calculated as the inverse of the threshold current and expressed as a percentage of the control response.

In these extracellular studies three types of experiments were conducted:

- (1) For testing the effects of a range of concentrations: After obtaining controls the muscle bundles were exposed for 30 minutes to each drug concentration and then tested. Each muscle was exposed to a sequentially increasing drug concentration.
- (2) For testing drug antagonism: The muscle was exposed for 30 minutes to each of 3 concentrations of the drug. The electrical responses of the muscle were tested at the start of the experiment (control) and immediately following each drug exposure. After each test the muscle was placed in the next highest drug concentration. Following this the muscle was allowed to recover, for about 90 minutes in drug-free Ringer's solution. Next another control was obtained and the muscle was exposed to solutions with the same drug concentrations plus a small concentration of an opiate antagonist and the responses tested as before.
- (3) For determining the time course of drug effects: After the control responses had been obtained, the muscle bundles were kept in a single low concentration of the drug for up to 300 minutes and the electrical responses tested at 30 minute

intervals. For comparison other muscle bundles were exposed to the same drug concentration for 90 minutes and then placed in a solution with the drug plus an opiate antagonist.

In all extracellular experiments, the initial control responses were obtained about 90 minutes after completion of dissection. This was the first response recorded in any experiment and drug additions were made subsequent to these recordings. The 90 minute wait before commencement of experiments was found to be essential for two reasons:

- (a) Control experiments (muscles maintained in Ringer's for up to 300 minutes and tested at 30 minute intervals) indicated that the effect of damage introduced by dissection took up to 90 minutes for recovery.
- (b) Muscles occasionally were infested by trematode parasites. The responses of such muscles are known to recover in 60-75 minutes if left in Ringer's (227).

F. Extracellular Stimulation and Intracellular Recording

In these experiments whole muscles were mounted horizontally, with their inner surface uppermost, in Ringer's solution in a Lucite bath. This was done because the superficial surface is covered with a dense layer of connective tissue which hinders the insertion of microelectrodes (8). For final mounting, the muscles were stretched approximately 10% of slack length over a glass post and then attached firmly to the hooks at the two ends in the Lucite bath (Figure 5).

The stretch subserved the following functions:

- (a) prevented damage to microelectrode tips and myofibre membrane upon movement of the muscle.

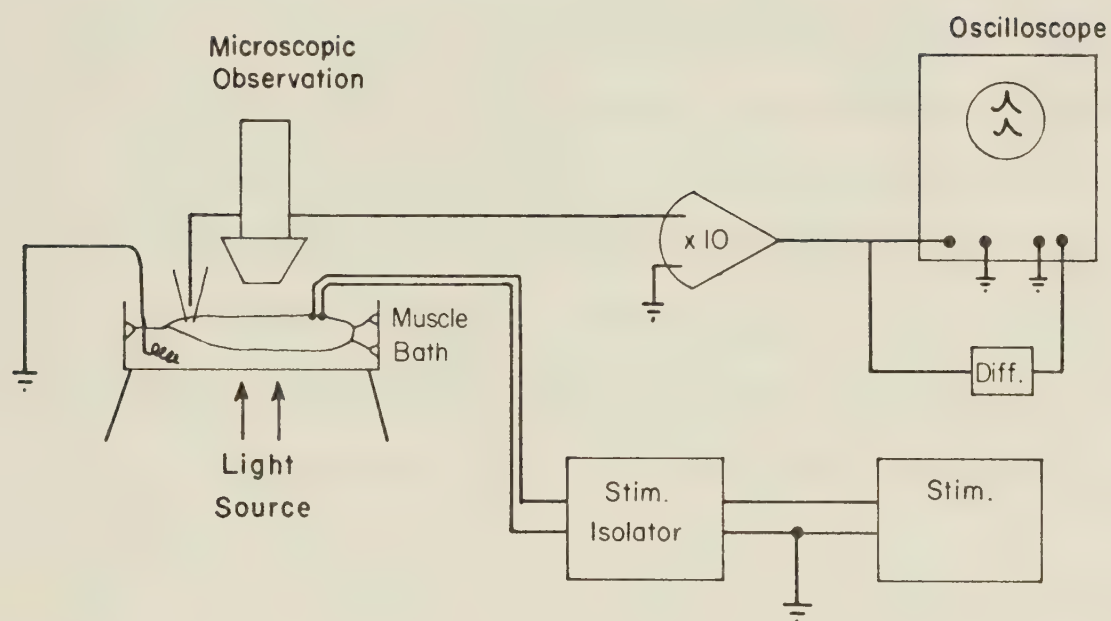


Figure 5: Diagrammatic illustration of the assembly used for extracellular stimulation and intracellular recording.

(b) minimized movement artifacts.

The Lucite bath containing the muscles was then placed on a Lucite stand underneath which there was a light source. This procedure allowed one to illuminate the muscles from below and view them from above with the aid of a binocular microscope (Wild-Heerbrugg, Switzerland).

Groups of fibres were stimulated by an extracellular bipolar wire filled pore electrode connected to the "stimulator". On bringing the electrode in contact with a portion of the surface of the muscle (with the aid of a micromanipulator), the fibres being stimulated could be identified by their movement in response to stimuli. The recording microelectrode was then lowered into one of these fibres for the purpose of recording the various electrical parameters of the myofibre. Two msec square-wave pulses were used throughout for stimulation of the fibres.

The recording electrode had an external tip diameter of less than 0.5 microns and was filled with 3M KCl. Only microelectrodes having resistances of about 15-35 M Ω were used. A solid state volt-ohm-microamp meter (Danameter, Dana Labs Inc., Irvine, California) was used to measure the resistance of the microelectrodes.

Movement of the recording microelectrode was controlled by a Prior micromanipulator. The electrode was held to a plastic arm attached to the manipulator by a chlorided silver wire used to connect the electrode to the amplifier. A small piece of rubber tubing around the silver wire held the shaft of the electrode, thus maintaining firm contact between the 3M KCl in the electrode and the wire. The reference electrode consisted of a chlorided silver wire, formed into a spiral, and placed in the solution bathing the muscle.

The maximal rate of rise of the action potential was determined by electrical differentiation. This was achieved by introducing a capacitance (20 pico farads) into the output of the D.C. amplifier. This differentiating circuit had a time constant of approximately 20 μ sec and provided an output voltage proportional to the rate of change of the input (219). Calibration was achieved by feeding a sawtooth wave into the input of the differentiating circuit. The bath fluid was not removed during experimental testing, which took about 10 minutes. Several myofibres were impaled at random and the resting membrane potentials, the normal and differentiated action potentials recorded. Oscilloscope records were captured on 35 mm film with a continuous recording camera and analyzed with the aid of a programmable calculator. Action potentials and their first derivatives (dV/dt) were recorded from several fibres over limited time periods (usually 5-15 min) throughout the course of an experiment. In over 90% of the periods recordings were from five to twelve fibres. Only when no excitable fibres were found was a value of 0 recorded for the time period. Otherwise inexcitable fibres were ignored in obtaining means.

G. Analysis

The positive ionic current (I_i) leaving the fibre during the falling phase of the action potential was calculated from the maximum rate of fall times the membrane capacitance (382). In making this calculation a specific membrane capacitance of $2.5 \mu\text{F}/\text{cm}^2$ was used. The slope and intercept was calculated for each set of data assuming a linear regression model (Figure 44) and from this information a maximum rate of fall calculated at a constant action potential

size of 130 mV. For the calculations with morphine or propoxyphene alone the results obtained in the 90 and 120 minute recording periods (Table 1) were used and for methadone the results from the 30 and 60 minute recording periods were used. In all cases the results from the last 2 recording periods with agonist plus antagonist were used for the results in Table 1c.

Where necessary, the significance of the difference between two means was assessed with Student's t-test; a level of significance of $P = 0.05$ was used.

In the course of this work the criteria listed by Kao and Nishiyama (218) for intracellular penetrations were adopted. These are:

- (a) sharp deflection on penetration to new d-c potential level (in the negative direction).
- (b) "cleanliness" of shift on reaching the new d-c level.
- (c) persistence of the new d-c level.
- (d) return to original baseline after withdrawing the electrode.

Table 1. Effects of some opiate drugs on the falling phase of the action potential spike.

Muscle	Max. rate of fall (V/sec) at AP = 130		ΔI_i (mA/cm ² , % reduction)	
	Control (a)	Agonist (b) + Antagonist (c)	a-b	b-c
Morphine 10 ⁻³ M	325	114.1	56.3	.145 (50.7)
	326	152.9	111.4	.104 (27.1)
	328	157.3	115.2	.105 (26.8)
	329	107.4	62.9	.111 (41.4)
	330	184.0	127.2	.142 (30.9)
	Means	143.1	94.6	.121 (35.4)
Methadone 10 ⁻⁴ M	125A	123.1	41.1	.205 (66.6)
	125B	104.1	52.0	.130 (50.0)
	126	100.9	51.2	.124 (49.3)
	127	103.9	63.1	.102 (39.3)
	128	149.6	132.3	.043 (11.6)
	129	136.2	128.5	.019 (5.6)
	130	147.7	115.5	.081 (21.8)
	Means	123.6	83.4	.101 (34.9)
Propoxyphene	318	158.9	107.1	.130 (32.6)
	319	100.7	50.5	.126 (49.9)
	320	101.5	56.7	.112 (44.1)
	321	180.1	127.1	.133 (29.4)
	322	151.3	107.2	.110 (29.1)
	Means	138.5	89.7	.122 (37.0)
* + 10 ⁻⁷ M Naloxone				
+ + 10 ⁻⁸ M Naltrexone				
			162.8*	-.089 (-28.1)
			139.7*	-.081 (-30.3)
			151.3	-.085 (-29.2)

V. RESULTS

Typical recordings made with extracellular bipolar stimulating and recording electrodes are shown in Figure 6. The top figure represents a voltage drop across a 50K resistor and such a trace was used to calculate the threshold current. The bottom figure represents a compound action potential. The distance between the stimulus artifact and the peak of the action potential was used in calculating the conduction velocity.

Figure 7 represents a typical action potential recorded with intracellular microelectrodes. The action potential was electrically differentiated. Recordings such as this were used in obtaining values for the resting membrane potential, the action potential, the overshoot potential, the maximum rate of rise and the maximum rate of fall.

Control Experiments

(a) Studies with Extracellular Electrodes

On exposure of the muscle strips to Ringer's solution, there was practically no change in excitability with periods up to 7 hours (Figure 8). The excitability of these muscle strips was tested immediately after dissection and at every thirty minutes thereafter. Usually 3 to 4 control responses of the "excitability" and the maximum amplitude of the compound action potential in Ringer's were obtained during each test. The mean of the initial control responses for each muscle preparation was used to calculate the per cent of control for the excitability and for the size of the compound action potential during subsequent treatment.

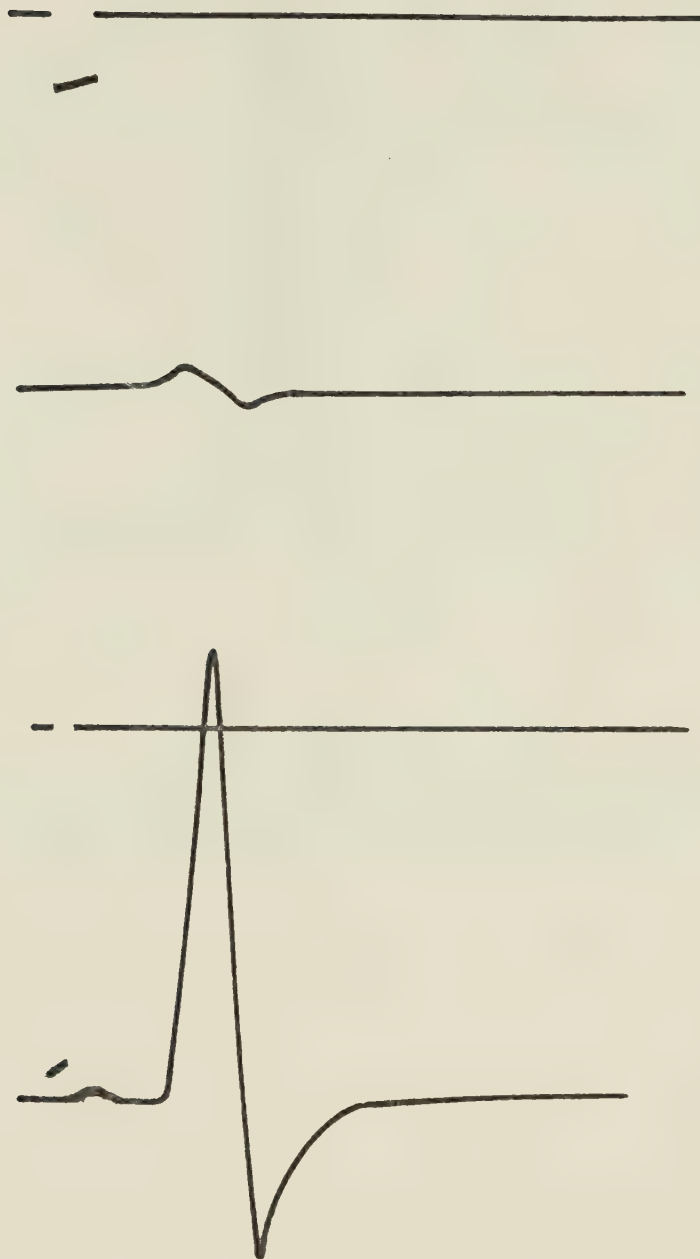


Figure 6: Typical recordings made with extracellular electrodes from frog sartorius muscle strips. Top figure represents a voltage drop across a 50K resistor. Bottom figure shows stimulus artifact and compound action potential.

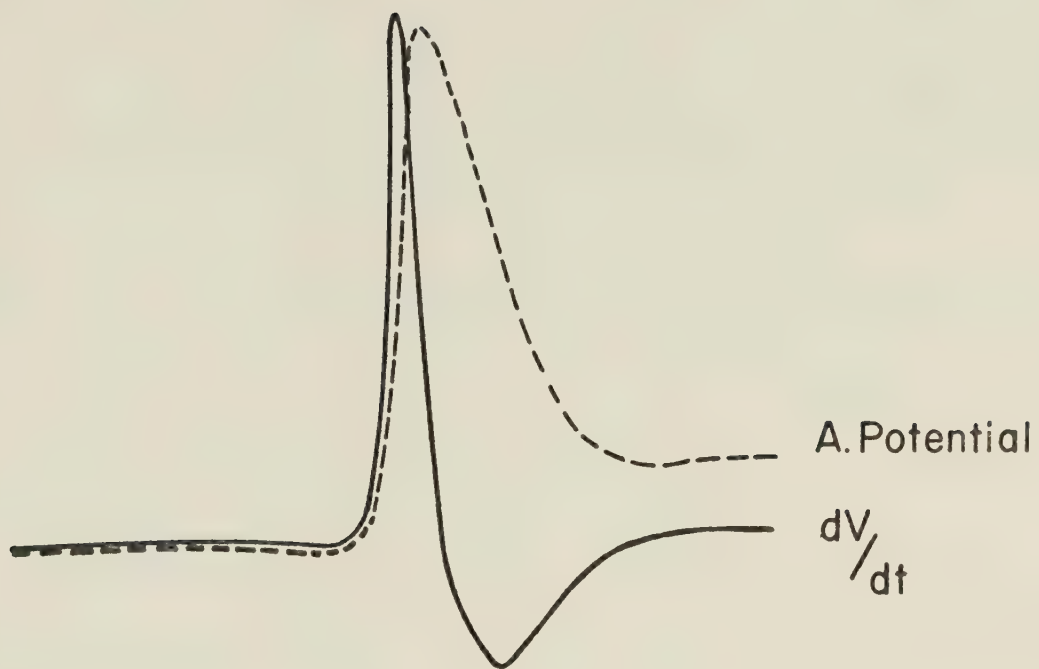


Figure 7: Typical electrically differentiated action potential recorded from frog sartorius muscle with inside-the-cell microelectrodes.

It can be seen that the size of the compound action potential decreased for the first 60 minutes after dissection. Concurrently, the threshold current needed to elicit this action potential increased. Thereafter, on continued exposure to Ringer's solution the magnitude of the compound action potential and the threshold currents returned to the initial level and remained relatively steady.

Thus, for all drug experiments, employing extracellular electrodes we adopted 90 minutes after dissection as a necessary waiting period before commencement of experimentation.

(b) Studies with Intracellular Electrodes

In these experiments, we utilized extracellular stimulation and intracellular microelectrode recording. Control extracellular studies had indicated that after dissection a wait of 90 minutes was necessary before commencement of experimentation. However, in "intracellular" studies, whole sartorii muscles rather than muscle bundles were employed and thus presumably any dissection injury was relatively small. Thus, a 60 minute wait was adopted as being a sufficient recovery time, before starting intracellular experiments.

Figure 9 depicts the effect on the resting membrane potential when the muscles were exposed to Ringer's solution. The excitability of these muscles was tested 60 minutes after dissection and for every 30 minutes thereafter. Usually 5-12 control responses of Ringer's were obtained during each test. The mean of the initial control responses for each muscle preparation was used to calculate the percent change in the excitability, during subsequent treatment. As seen from Figure 9, on exposure to Ringer's solution the resting membrane

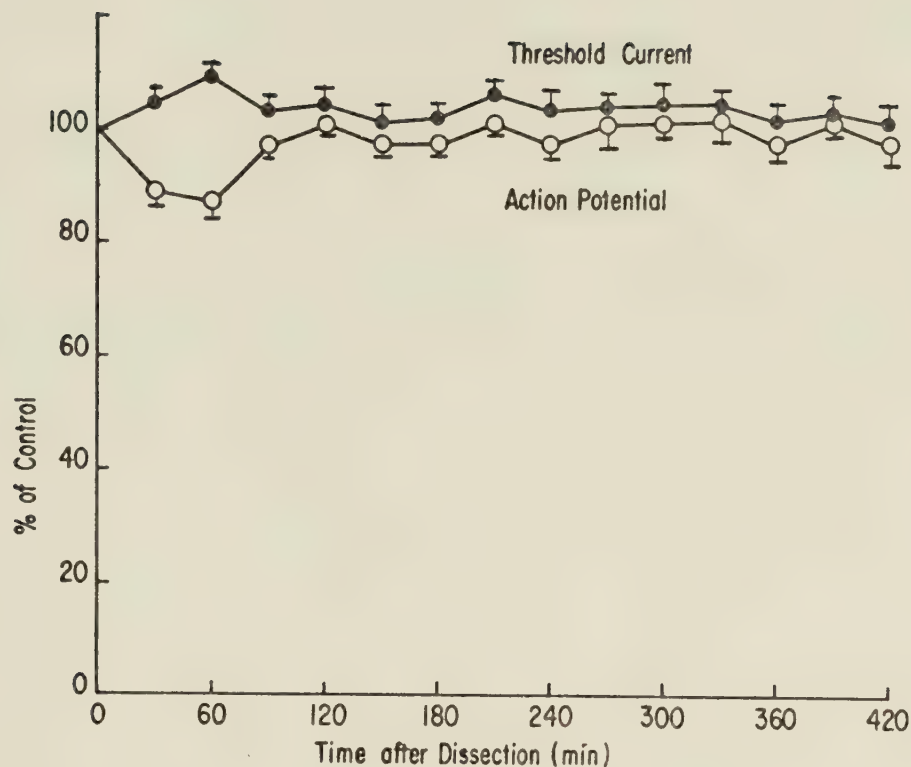


Figure 8: Control study of the maximum amplitude of the compound action potential and the threshold current with frog's sartorius muscles kept in Ringer's solution. Extracellular recording. Means \pm standard error (S.E.) recorded for each test period. The results obtained in each experiment were calculated separately, the values at 30 minute intervals obtained from the results, the means \pm S.E.M. calculated and used to produce the graph shown. Number of muscles (n) = 4.

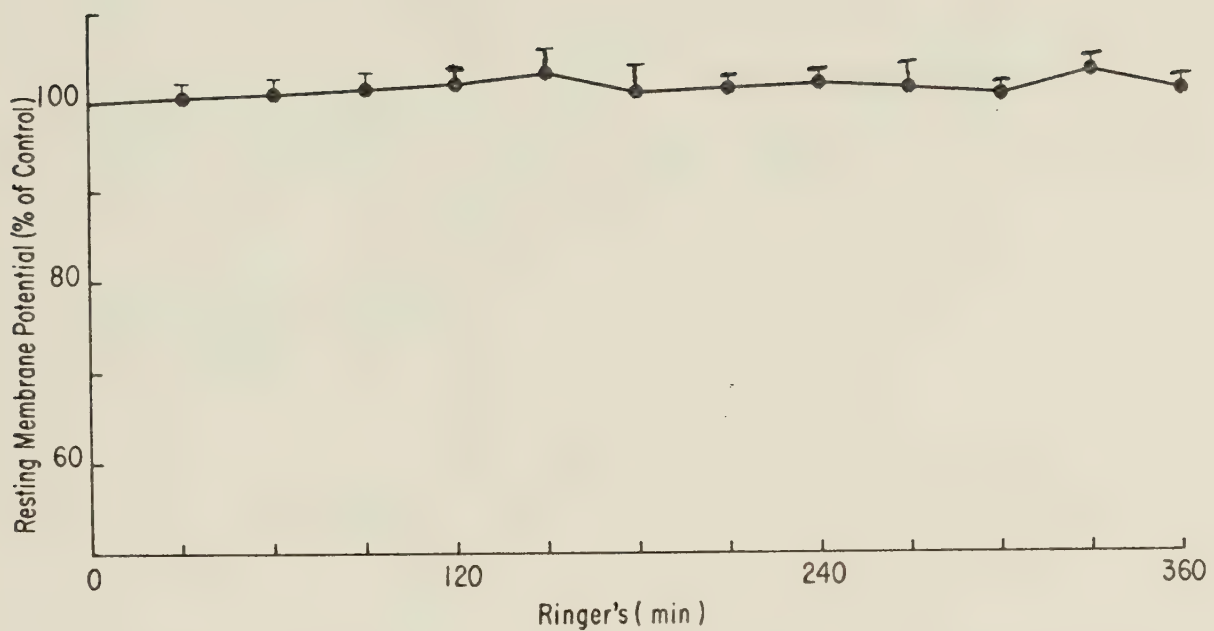


Figure 9: Control study of the resting membrane potential recorded with intracellular microelectrodes. Muscles kept in Ringer's, $n=3$ muscles. For each muscle 6 to 10 resting potentials were recorded at each test and a mean for the muscle calculated. Means \pm S.E.M calculated from the muscle means are plotted.

potential (RMP) remained steady and relatively unaltered. This indicates that the experimental conditions employed did not alter or affect the RMP with time.

As shown in Figure 10, there was no significant change in the action potential maximum rate of rise during exposure to Ringer's for up to 6 hours. Similarly the results presented in Figure 11 show that there was no significant change in the maximum rate of fall during exposure to Ringer's solution. We thus conclude that the experimental conditions employed in this study, do not by themselves alter excitability parameters in frog sartorius muscles.

Extracellular Studies

(a) Propoxyphene - HCl

Exposure to increasing concentrations of (30 minutes at any concentration) propoxyphene - HCl increased the threshold current and decreased the action potential amplitude and conduction velocity (Figure 12). This effect increased with increasing concentrations of propoxyphene.

Figure 13(a) shows the effect of increasing concentrations of propoxyphene on the maximum amplitude of the compound action potential. When the muscles were allowed to recover in Ringer's after treatment with the highest concentration of propoxyphene, on subsequent exposure to the same concentrations of propoxyphene plus a low concentration of naloxone there was a significant increase in the compound action potential. Thus, naloxone at concentration $3 \times 10^{-7}M$ antagonized the depressant effects of propoxyphene HCl. The effects on the threshold current and conduction velocity from

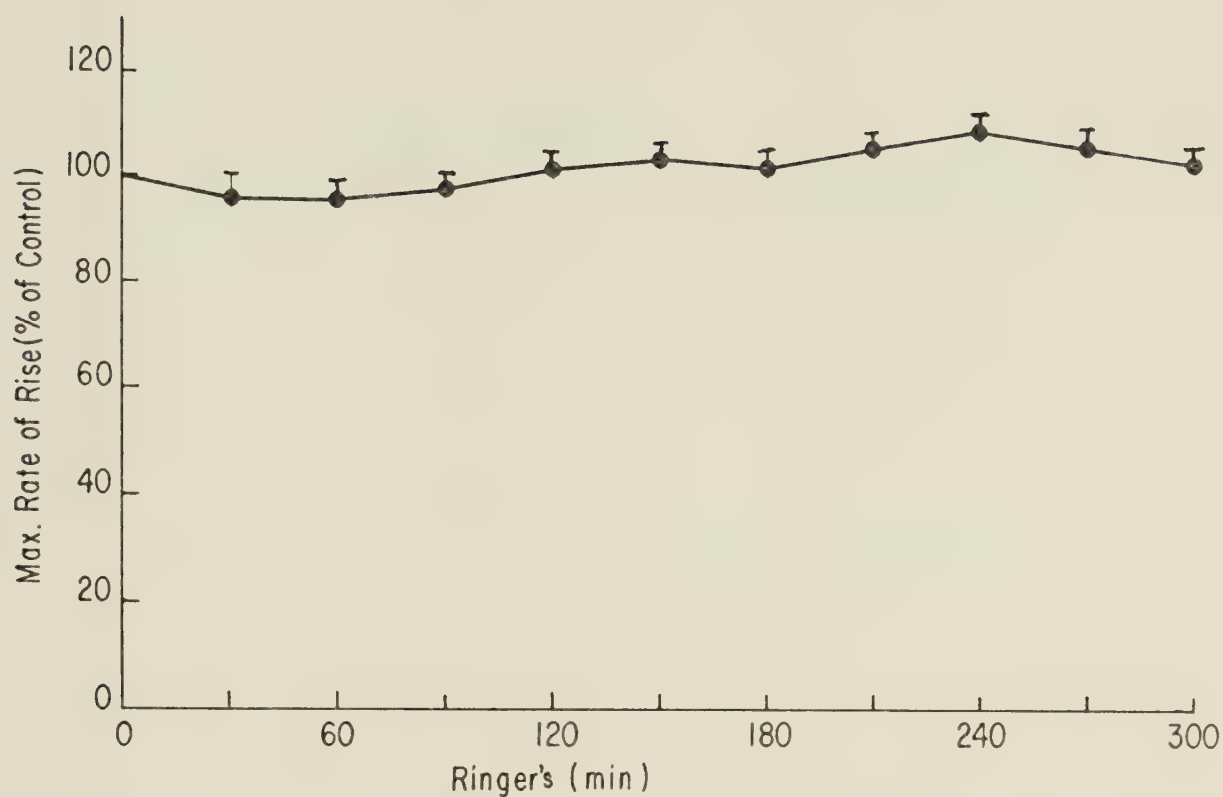


Figure 10: Control study of the action potential maximum rate of rise recorded intracellularly. Muscles kept in Ringer's, $n=3$ muscles. For each muscle 6 to 10 recordings were made at each test and a mean for the muscle calculated. Means \pm S.E.M. calculated from the muscle means are plotted.

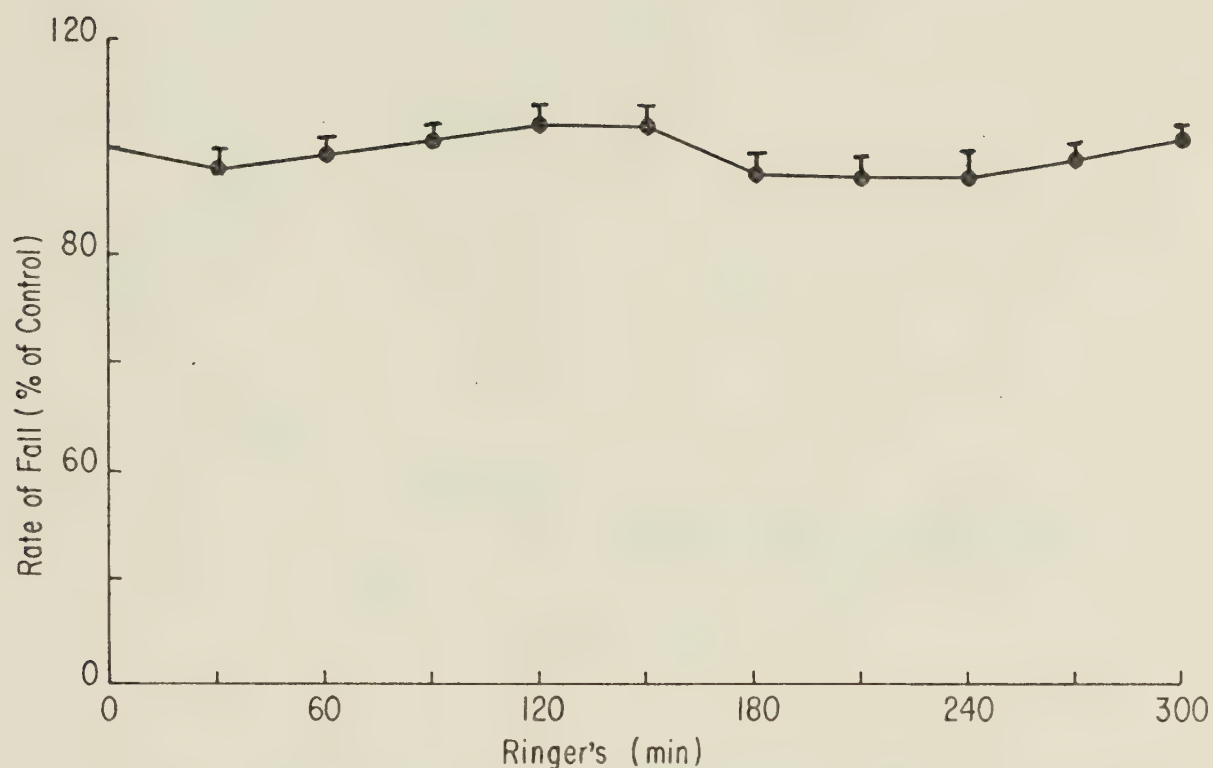


Figure 11: Control study of the action potential maximum rate of fall recorded intracellularly. Muscles kept in Ringer's, $n = 3$ muscles. For each muscle 6 to 10 recordings were made at each test and a mean for the muscle calculated. Means \pm S.E.M. calculated from the muscle means are plotted. The results obtained in Figures 9, 10, 11 were obtained in a single set of experiments.

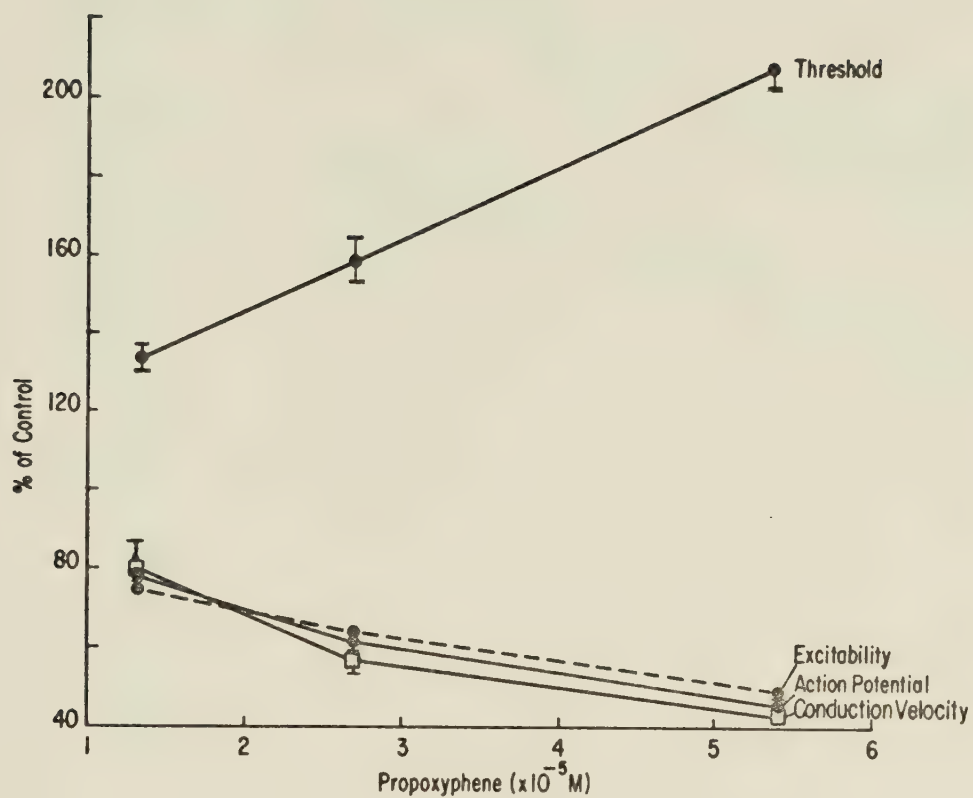


Figure 12: Effects of various propoxyphene concentrations on extracellularly recorded action potentials of frog's sartorius muscle strips. Means \pm S.E.M.: $n = 5$.

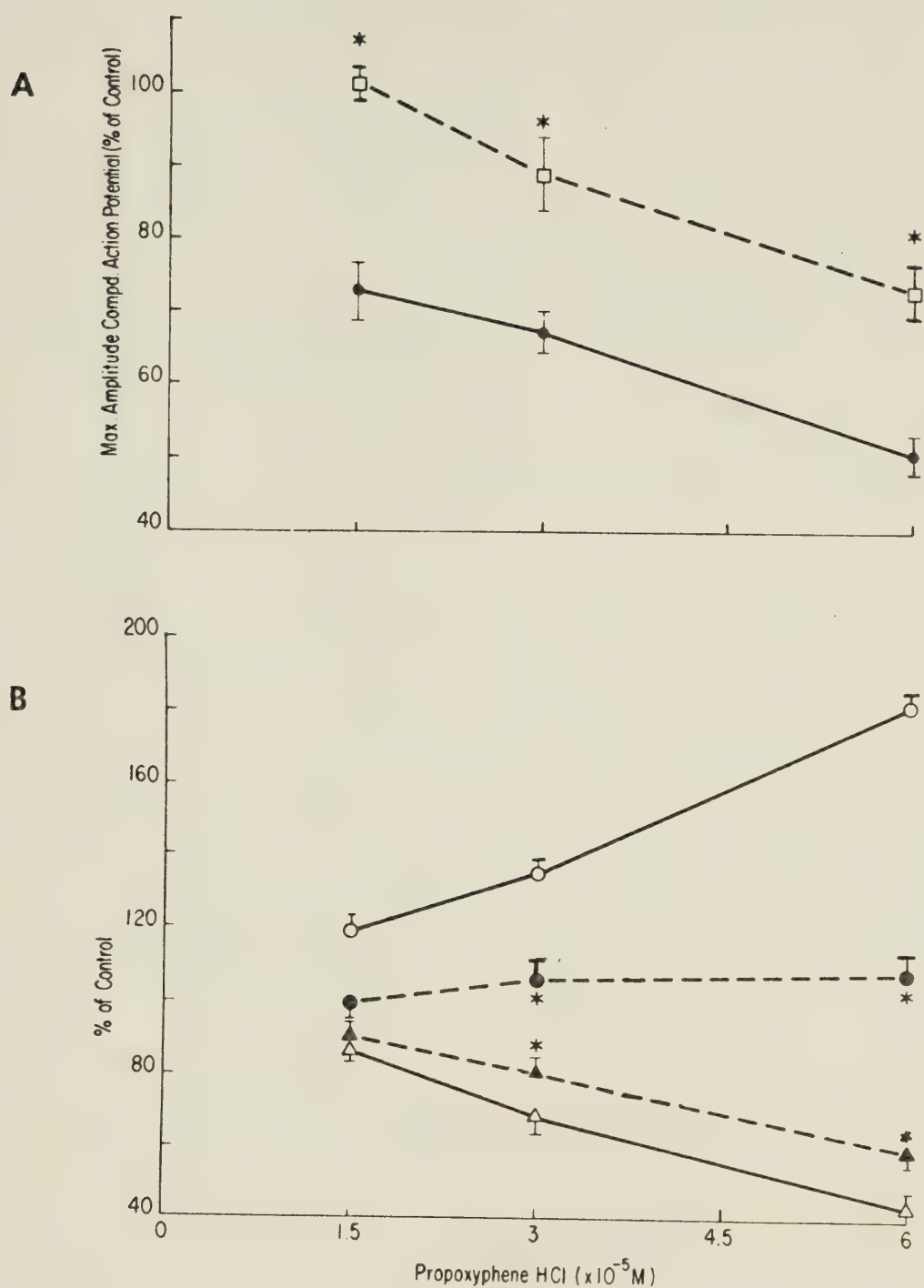


Figure 13: Naloxone (3×10^{-7} M) antagonism of the depressant effects of propoxyphene on the extracellularly recorded action potential of frog's sartorius muscle strips. In A, mean \pm S.E.M.: ●—●, propoxyphene (P) alone; □----□, P plus naloxone (N). In B, mean \pm S.E.M.: ○—○, threshold current - P alone; ●----●, threshold current - P + N; △—△, conduction velocity - P alone; ▲----▲, conduction velocity - P + N. *, means with and without naloxone significantly different at $p = 0.05$. Results from experiments with 5 muscle strips all exposed to the 6 drug combinations. A paired t-test was conducted.

the same experiments are shown in Figure 13(b). Naloxone antagonized the effects of propoxyphene, but under the conditions of these experiments this antagonism was statistically significant only at the two higher propoxyphene concentrations.

It was found that during a prolonged exposure to a single low concentration of propoxyphene there was a progressive decline in the maximum amplitude of the compound action potential with time (Figure 14). In similar experiments, it was found that when a low concentration of naltrexone was added to the bathing medium at about 120 minutes, the depression in the maximum amplitude of the compound action potential elicited by propoxyphene HCl, was significantly antagonized.

As shown in Figure 15 a prolonged exposure to a single low concentration of propoxyphene produced a progressive decline in the maximum amplitude of the compound action potential. A low concentration ($10^{-7}M$) of meperidine added to the bathing medium at 60 minutes did not antagonize the depression in the maximum amplitude of the compound action potential elicited by propoxyphene.

(b) Methadone - HCl

As shown in Figure 16, methadone elevated the threshold current and concurrently depressed the action potential, the excitability and conduction velocity. This effect became more pronounced with increasing concentrations of methadone.

Figure 17 illustrates the effect of methadone on the conduction velocity. As indicated above, there was an increasing depression in conduction velocity with increasing concentrations of methadone. However, when these same muscle strips were allowed to recover in Ringer's

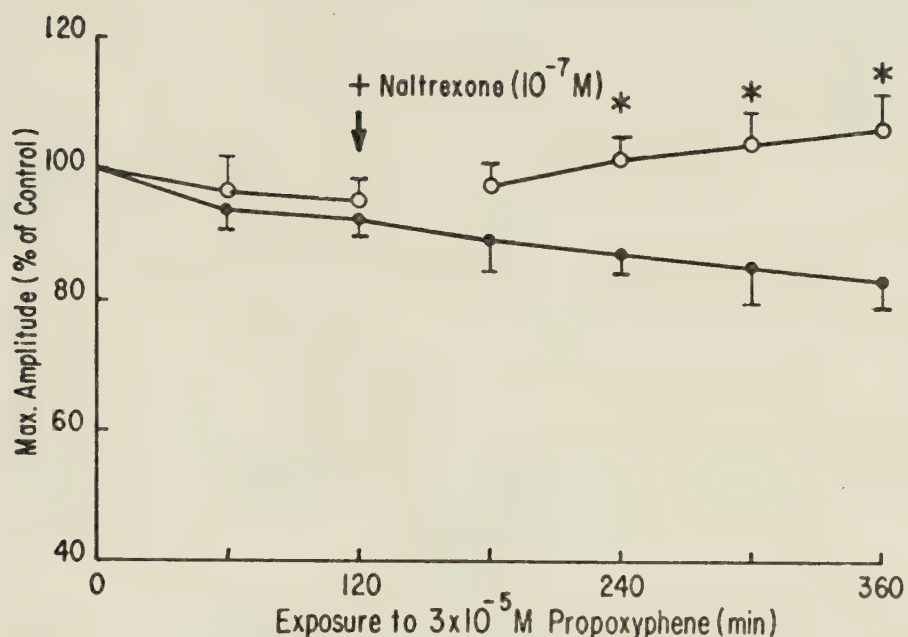


Figure 14: Effect of a low propoxyphene concentration (3×10^{-5} M) on the maximum amplitude of the compound action potential of frog's sartorius muscle strips and the antagonistic effect of naltrexone (10^{-7} M). Results from experiments with 6 muscles; 3 with propoxyphene alone (●) and 3 with propoxyphene plus naltrexone (○). All muscles exposed to propoxyphene at time 0. Naltrexone added between 100-120 minutes. The results obtained and plotted as in Figure 8. Means \pm S.E.M. *, means with and without naltrexone significantly different at $p = 0.05$. An unpaired t-test was conducted.

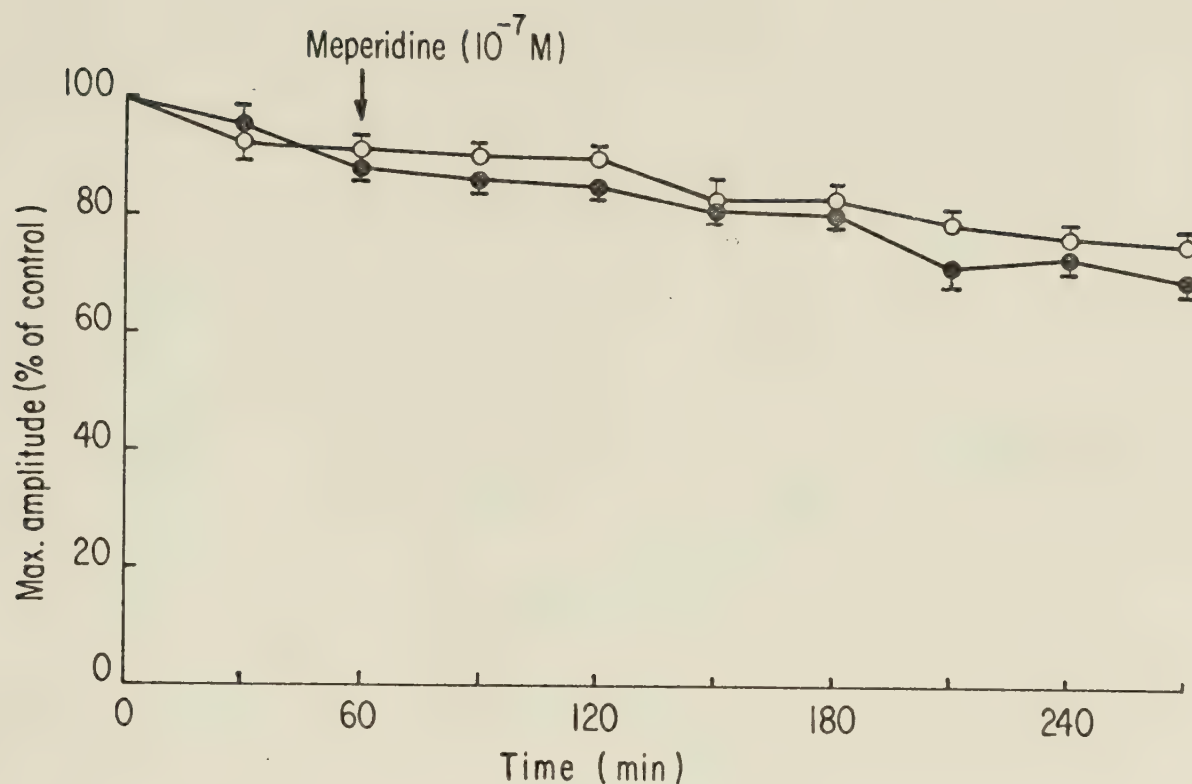


Figure 15: Lack of an antagonistic effect of meperidine on the depressant effects of propoxyphene (10^{-5} M) on the extracellularly recorded compound action potential of frog's sartorius muscle strips. Means \pm S.E.M. o, propoxyphene alone ($n = 3$); ●, propoxyphene plus meperidine ($n = 3$). All muscles exposed to propoxyphene at time 0. Meperidine added at 60 minutes. An unpaired t-test was conducted.

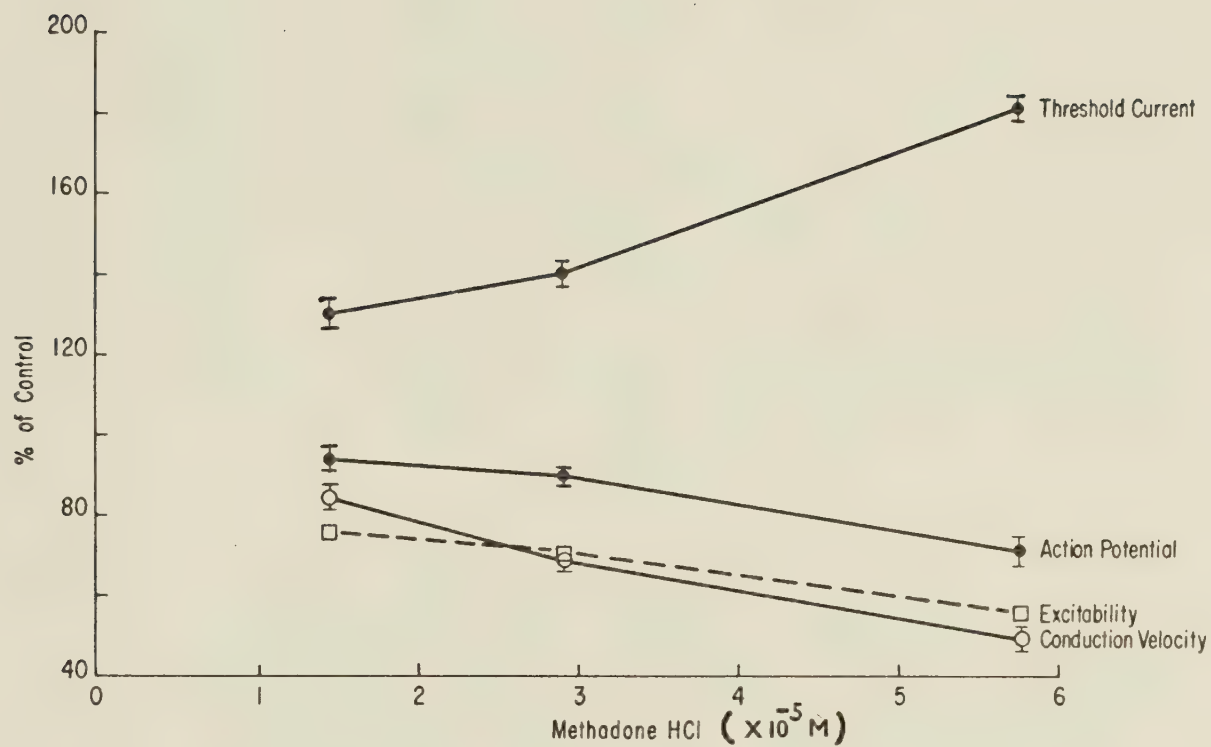


Figure 16: Effects of various concentrations of methadone on extra-cellularly recorded action potentials of frog sartorius muscle strips. Results calculated and plotted as in Figure 8 $n = 6$, means \pm S.E.M.

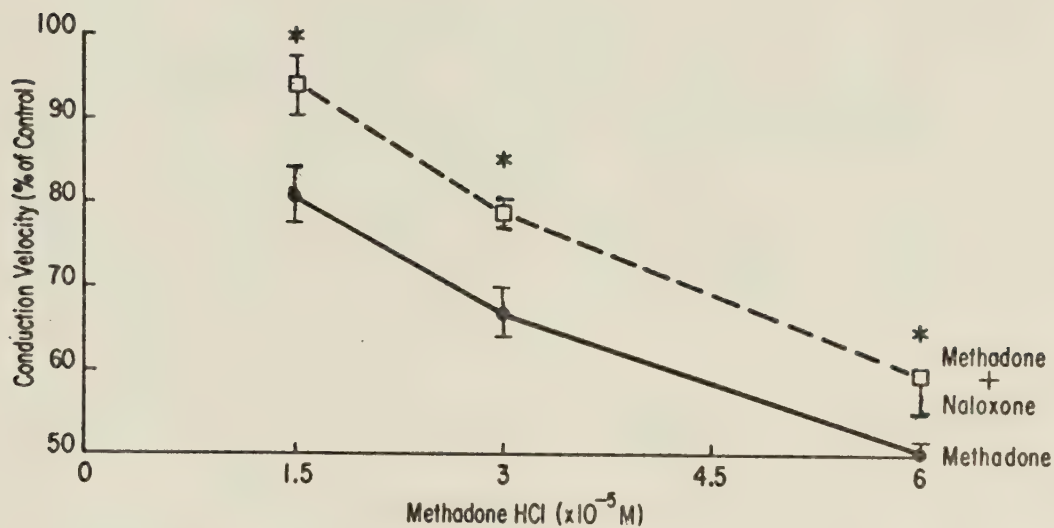


Figure 17: Naloxone (10^{-7} M) antagonism of the depressant effects of methadone on the conduction velocity of frog's sartorius muscle strips. Means \pm S.E.M. ●, methadone alone; □, methadone plus naloxone. Results from experiments with 3 muscles all exposed to the 6 drug combinations. Results plotted as in Figure 8. *, means with and without naloxone significantly different at $p = 0.05$. A paired t-test was conducted.

and then re-exposed to the same concentration of methadone, it was observed that naloxone significantly antagonized the depressant effect of methadone.

When muscles were exposed to a single low concentration of methadone (Figure 18), the maximum amplitude of the compound action potential was increasingly depressed with time. However, in similar experiments, on addition of naltrexone ($10^{-7}M$) at approximately 90 mins to the bathing medium, we found that this low concentration of naltrexone could significantly antagonize the depressant effect of methadone on the compound action potential.

In general, the effects produced by methadone on the various excitability parameters of the frog skeletal muscle were qualitatively very similar to those produced by propoxyphene when using the extracellular electrode techniques.

(c) Morphine - HCl

Figure 19 illustrates the effects of morphine on the threshold current, the action potential and the conduction velocity in the frog sartorius muscle. The threshold current increased and the action potential and conduction velocity decreased upon exposure to morphine. This effect became more pronounced with increasing concentrations of morphine HCl.

The effect of morphine on the conduction velocity is shown in Figure 20. There was a marked depression in the conduction velocity with increasing concentrations of morphine HCl. When these same muscles were allowed to recover in drug free Ringer's and then exposed to the same concentrations of morphine plus a small concentration of

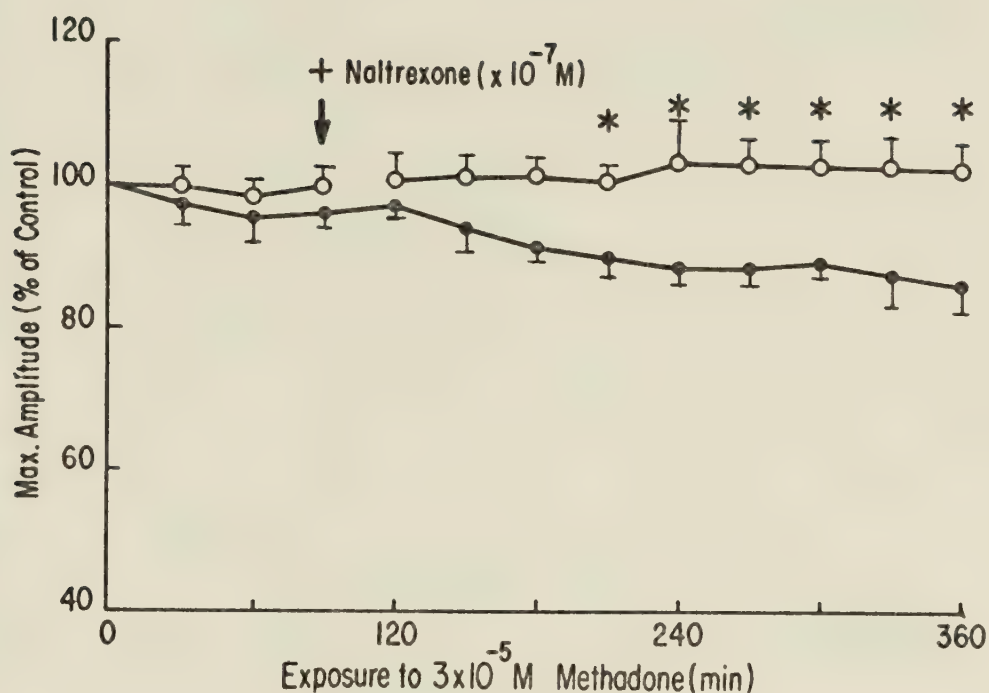


Figure 18: Effect of a low methadone concentration (3×10^{-5} M) on the maximum amplitude of the compound action potential of frog's sartorius muscle strips and the antagonistic effect of naltrexone (10^{-7} M). Results from experiments with 6 muscles; 3 with methadone alone (○) and 3 with methadone and methadone plus naltrexone (●). All muscles exposed to methadone at time 0. Naltrexone added between 100-120 minutes. The results obtained and plotted as in Figure 8. Means \pm S.E.M. *, means with and without naltrexone significantly different at $p = 0.05$. An unpaired t-test was conducted.

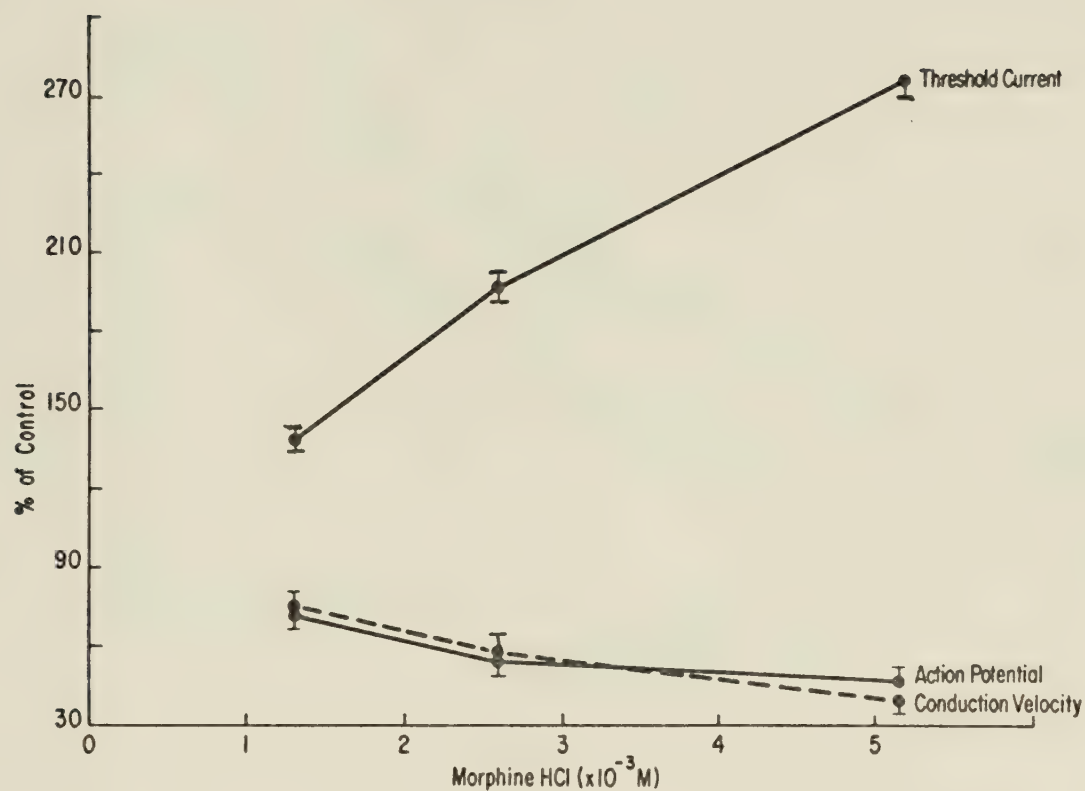


Figure 19: Effects of various concentrations of morphine hydrochloride on extracellularly recorded maximum amplitude of compound action potentials in frog sartorius muscle strips. $n = 6$ means \pm S.E.M. Results obtained and plotted as in Figure 8.

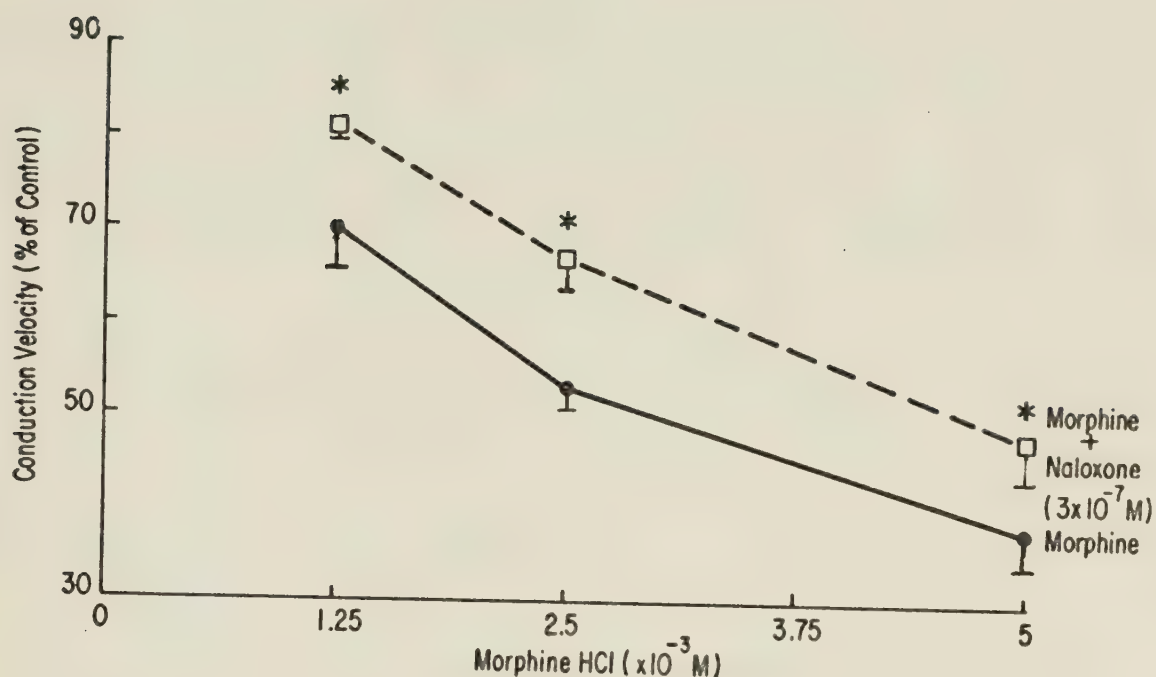


Figure 20: Naloxone (3×10^{-7} M) antagonism of the depressant effects of morphine hydrochloride on the compound action potential of frog's sartorius muscle strips. Means \pm S.E.M. \bullet , morphine alone; \square , morphine plus naloxone. $n = 3$ muscles. All 3 muscles exposed to the 6 drug combinations. *, means with and without naloxone, significantly different at $p = 0.05$. A paired t-test was conducted.

naloxone, the addition of naloxone significantly antagonized the depressant effects of morphine on the conduction velocity.

Figure 21 shows the effect produced by a single concentration of morphine on the compound action potential. With time, the maximum amplitude of the compound action potential became increasingly depressed. When a small concentration of naltrexone was added at approximately 90 minutes to the bathing medium, it significantly antagonized the depressant effects of morphine on the compound action potential. In contrast to the results observed with propoxyphene and methadone the antagonistic effect only became apparent 120 minutes after the addition of the antagonist.

(d) Meperidine - HCl

The effects of 3 meperidine concentrations (Figure 22) were tested by themselves and in the presence of naltrexone. With increasing concentrations the effect of meperidine became more pronounced. Naltrexone antagonized the effects of the 2 lower doses but not those of the largest dose of meperidine.

In another type of experiment muscle bundles were continuously exposed to a low concentration of meperidine (10^{-4} M) either with or without naltrexone (10^{-7} M), Figure 23. In all tests, after the initial control responses were obtained, the muscles were placed in a solution with only meperidine at time 0. In 4 experiments the muscles remained in this solution except when testing the electrical response. In the rest of the experiments the muscles were placed in a solution containing the same meperidine concentration plus naltrexone at time 60 minutes and remained in this solution. With this meperidine concentration

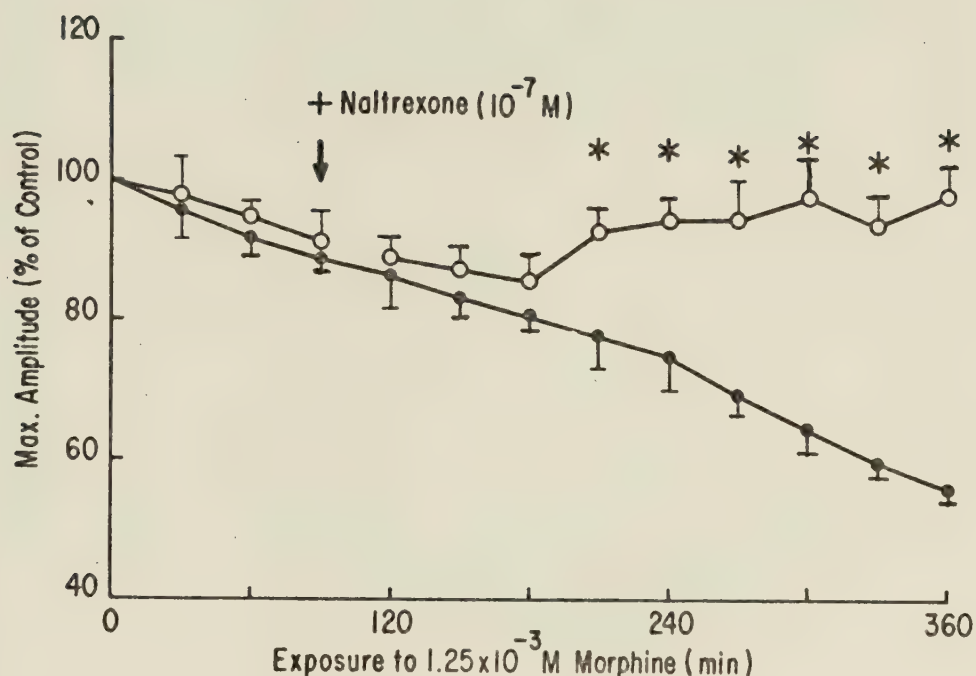


Figure 21: Effect of a single morphine HCl concentration (1.25×10^{-3} M) on the maximum amplitude of the compound action potential of frog's sartorius muscle strips and the antagonistic effect of naltrexone (10^{-7} M). Results from experiments with 6 muscles; 3 with morphine alone (●) and 3 with morphine and morphine plus naltrexone (○). All muscles exposed to morphine at time 0. Naltrexone added between 70 to 90 minutes. The results obtained and plotted as in Figure 8. Means \pm S.E.M. *, means with and without naltrexone significantly different at $p = 0.05$. An unpaired t-test was conducted.

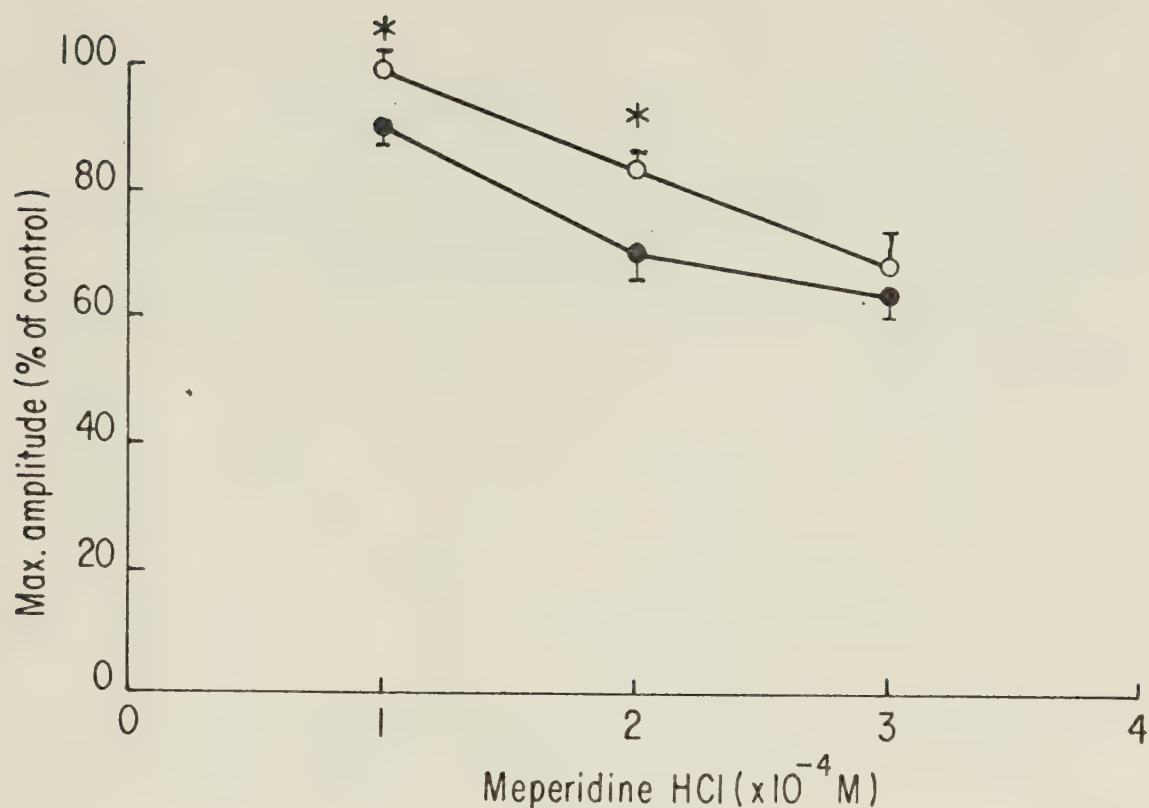


Figure 22: Naltrexone (10^{-7} M) antagonism of the meperidine depression of the maximum amplitude of the compound action potential of frog's sartorius muscles. Means \pm S.E.M.: ●, meperidine alone; ○, meperidine plus naltrexone ($n = 3$). Muscles all exposed to the 6 drug combinations. *, means with and without naltrexone significantly different at $p = 0.05$. A paired t-test was conducted.

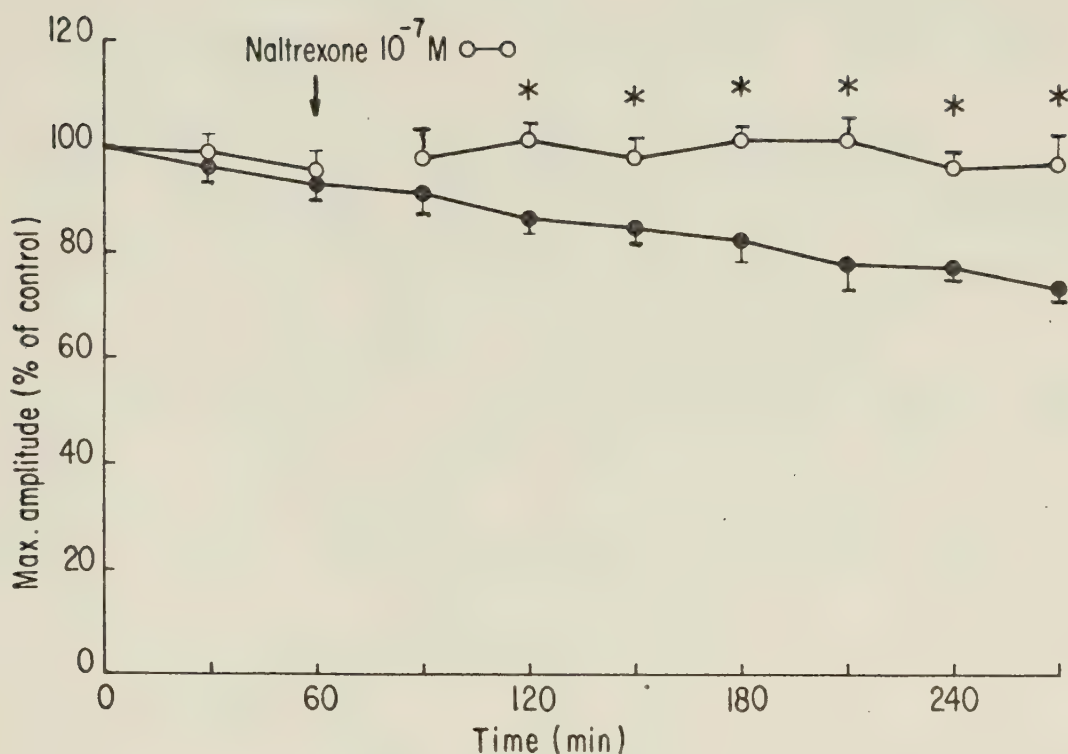


Figure 23: Effect of a relatively low meperidine concentration ($10^{-4}M$) on the maximum amplitude of the compound action potential of frog's sartorius muscles and the antagonistic effect of naltrexone. Results from experiments with 6 muscles; means \pm S.E.M. 3 with meperidine alone (\bullet) and 3 with meperidine plus naltrexone (\circ). All muscles exposed to meperidine at time 0. Naltrexone added at 60 minutes. *, means with and without naltrexone significantly different at $p = 0.05$. An unpaired t-test was conducted.

(10^{-4}M) there was a slow but gradual decrease in the compound action potential. When naltrexone was added to the bathing solution at 60 minutes, the depression in the amplitude of the compound action potential due to meperidine was significantly antagonized.

Similar experiments conducted with meperidine and dextromethorphan and meperidine and propoxyphene are shown in Figures 24 and 25 respectively. In both cases single low concentrations (10^{-4} or $2 \times 10^{-4}\text{M}$) of meperidine produced a progressive decline in the maximum amplitude of the compound action potential, which could not be antagonized by adding at 60 minutes either dextromethorphan (10^{-7}M) or propoxyphene (10^{-7}M) to the solution bathing the muscles.

(e) Naloxone - HCl

The effects of various concentrations of naloxone on excitability are shown in Figure 26.

With 30 minute exposures up to a concentration of $3 \times 10^{-7}\text{M}$, naloxone did not produce any observable depressant effects. However, with higher concentrations, there was an elevation in the threshold current and a concurrent decrease in the conduction velocity and the maximum amplitude of the compound action potential. This indicated that naloxone is a partial agonist with a very low intrinsic activity, in this preparation.

The effects of a low concentration of naltrexone (another opiate antagonist) on the agonist effects of high naloxone concentrations were investigated. The results of this study are shown in Figure 27. With increasing concentrations of naloxone, there was an increasing elevation in the threshold current and a decrease in the maximum amplitude of the compound action potential. When these muscles were allowed to

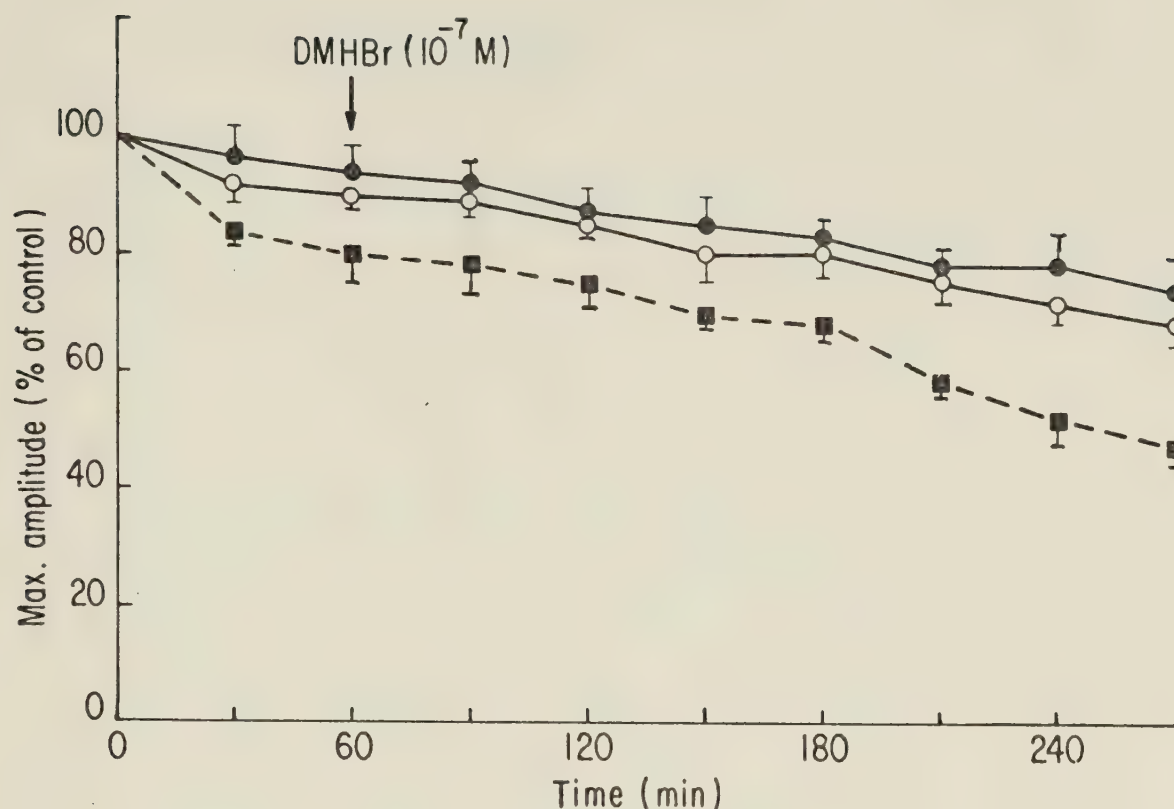


Figure 24: Effect of low meperidine concentrations, (●, 10^{-4} M; and ■, 2×10^{-4} M, respectively) on the maximum amplitude of the compound action potential of frog's sartorius muscles and the lack of antagonistic effect of dextromethorphan (DMHBr) on the depression elicited by 10^{-4} M meperidine (○). $n = 9$ muscles; 3 with meperidine 10^{-4} M (●), 3 with meperidine 2×10^{-4} M (■) and 3 with meperidine (10^{-4} M) and meperidine plus DMHBr (○). All muscles exposed to meperidine at time 0. DMHBr added at 60 minutes; means \pm S.E.M.. An unpaired t-test was conducted.

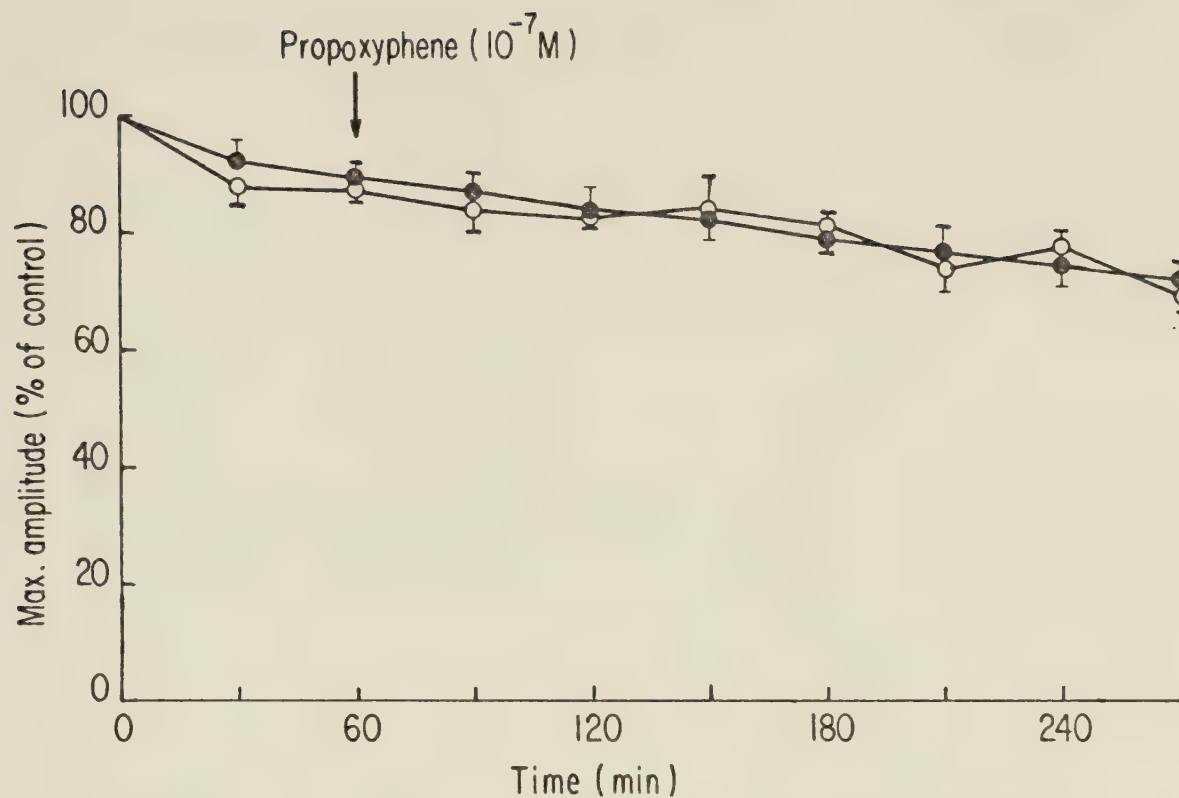


Figure 25: Lack of an antagonistic effect of propoxyphene on the depressant effects of meperidine (10^{-4} M) on the extra-cellularly recorded compound action potential of frog's sartorius muscle strips. Means \pm S.E.M. \bullet , meperidine alone ($n = 3$). \circ , meperidine plus propoxyphene ($n = 3$). All muscles exposed to meperidine at time 0. Propoxyphene added at 60 minutes. An unpaired t-test was conducted.

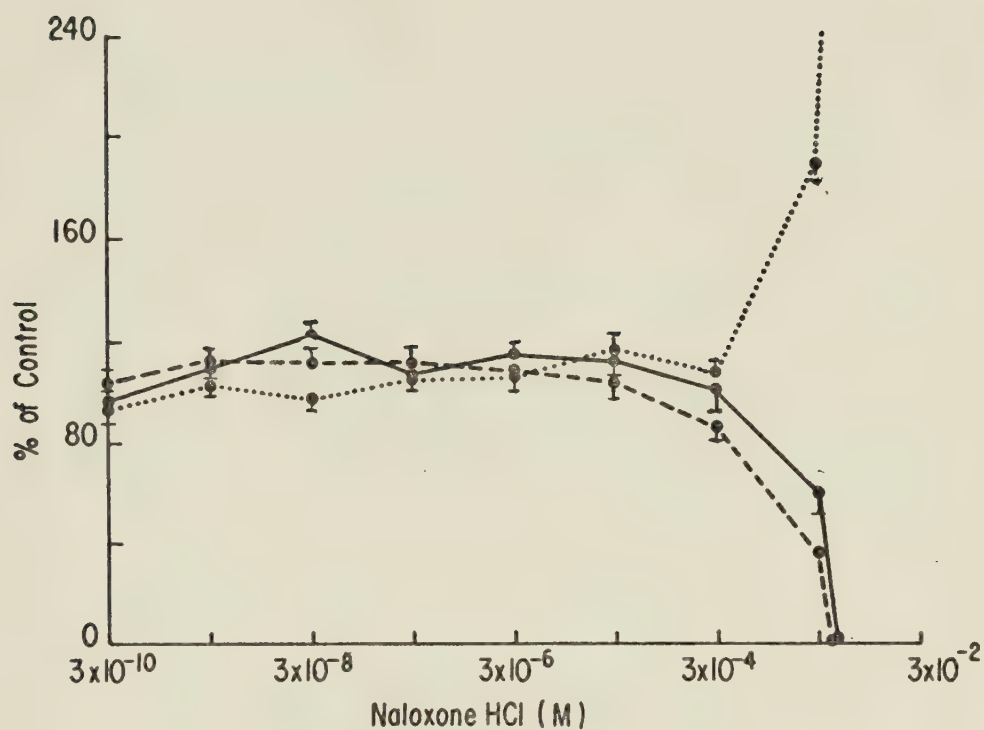


Figure 26: Effects of various naloxone concentrations on extra-cellularly recorded compound action potentials of frog sartorius muscle strips. $n = 3$, means \pm S.E.M. Results obtained and plotted as in Figure 8. $\bullet \dots \bullet$, threshold current; $\bullet \text{---} \bullet$, maximum amplitude of compound action potential; $\bullet \text{---} \bullet$, conduction velocity.

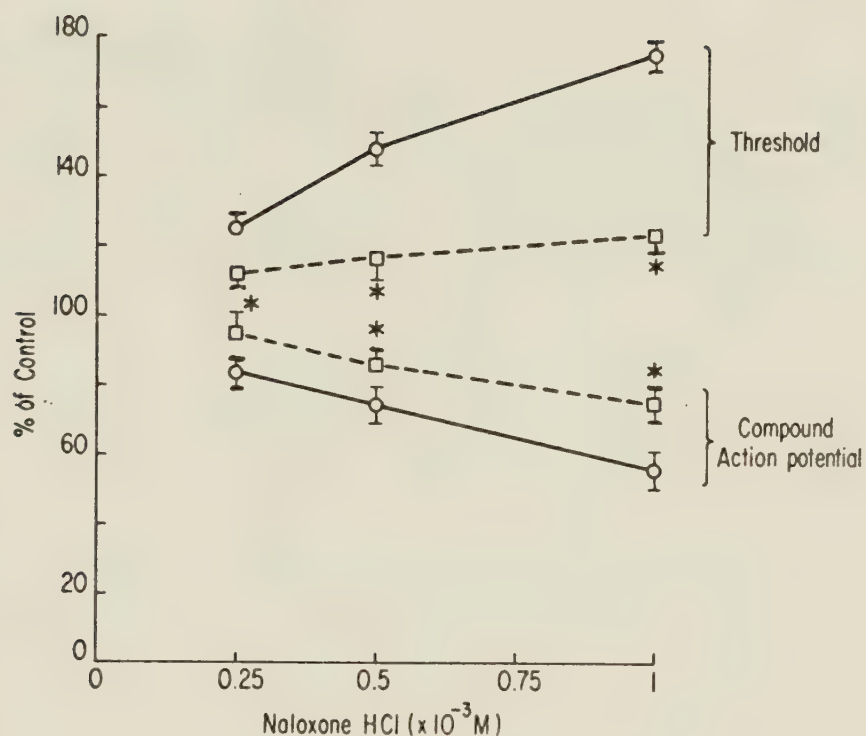


Figure 27: Antagonistic effect of a low concentration (10^{-7} M) of naltrexone on the action potential depression produced by high concentrations of naloxone, in frog sartorius muscle strips. Extracellular recordings. \circ — \circ , naloxone alone; \square — \square , naloxone plus naltrexone (10^{-7} M). Means \pm S.E.M. $n = 3$. All 3 muscles exposed to the 6 drug combinations. *, means with and without naltrexone significantly different at $p = 0.05$. A paired t-test was conducted.

recover in drug-free Ringer's and subsequently exposed to the same concentrations of naloxone plus a low concentration of naltrexone (10^{-7}M), naltrexone significantly antagonized the depressant effects of high concentrations of naloxone.

(f) Naltrexone - HCl

Figure 28 shows the effects of a range of naltrexone concentrations on conduction velocity. Up to a concentration of 10^{-5}M , naltrexone (30 minute exposures) was without any marked effects on conduction velocity. However, at higher concentrations, naltrexone produced excitability depression. Thus, naltrexone like naloxone, was found to be a partial agonist with a low intrinsic activity.

The depressant effects of high concentrations of naltrexone could not be antagonized by employing antagonistic concentrations of naloxone. This is shown in Figure 29. With increasing concentrations of naltrexone there was an elevation in the threshold current and a concurrent decrease in the maximum amplitude of the compound action potential. When these muscles were allowed to recover in drug-free Ringer's solution and then reexposed to the same concentrations of naltrexone plus an antagonistic concentration of naloxone, it was observed that naloxone did not antagonize the depressant effects of high concentrations of naltrexone.

(g) Procaine - HCl

In Figure 30 are shown the effects of procaine on excitability in the frog sartorius muscle. With increasing concentrations, procaine's depressant effects became more pronounced.

Figure 31, shows the effects of three concentrations of procaine with and without antagonistic concentrations of naloxone on the maximum

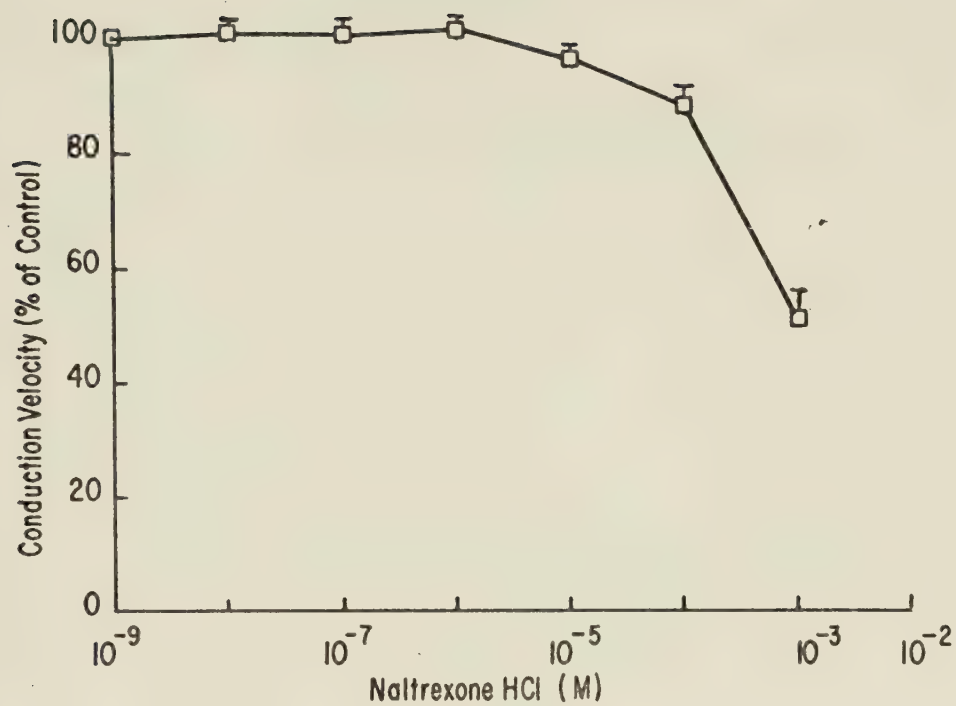


Figure 28: Effect of various naltrexone concentrations on the conduction velocity of frog's sartorius muscle strips. $n = 6$ muscles, means \pm S.E.M. Means obtained as described in Figure 8.

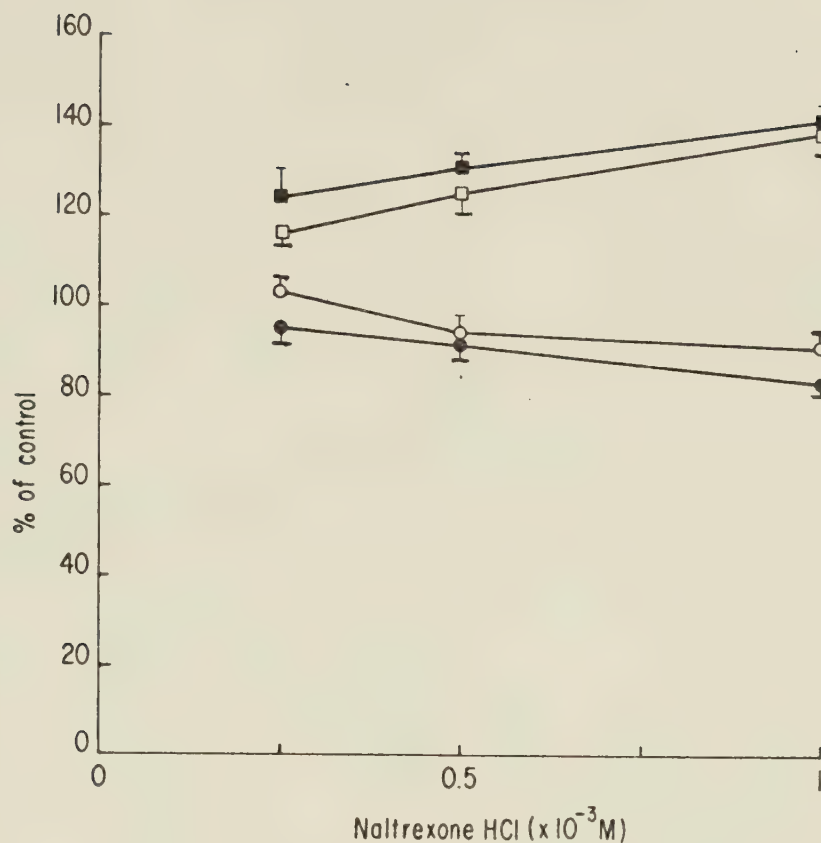


Figure 29: Lack of an antagonistic effect of naloxone (10^{-7} M) on the depressant effects of naltrexone, on the extracellularly recorded compound action potential of frog's sartorius muscle strips. Means \pm S.E.M. Threshold current: ■, naltrexone alone; □, naltrexone plus naloxone. Compound action potential: ●, naltrexone alone; ○, naltrexone plus naloxone. $n = 3$. All muscles exposed to the 6 drug combinations. A paired t-test was conducted.

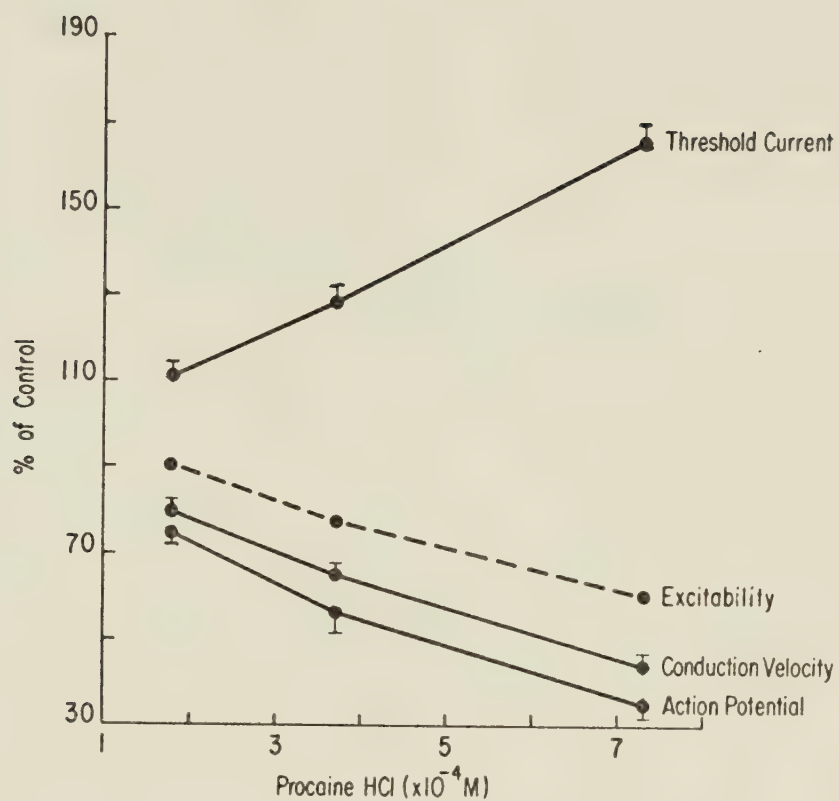


Figure 30: Effects of various concentrations of procaine hydrochloride on extracellularly recorded maximum amplitude of compound action potentials in frog sartorius muscle strips. $n = 6$, means \pm S.E.M. Results obtained and plotted as in Figure 8.

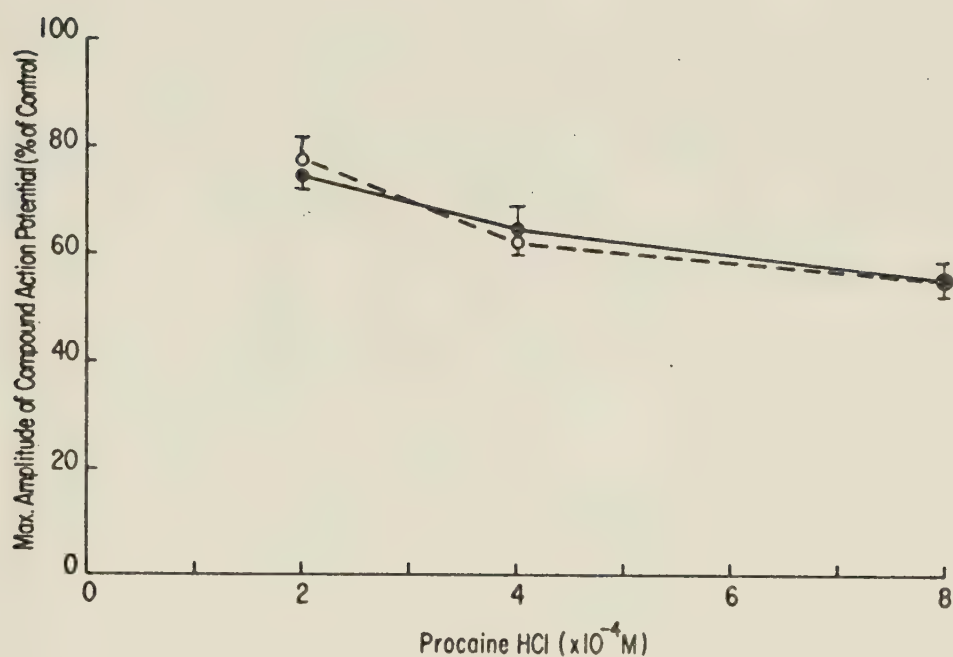


Figure 31: Lack of an antagonistic effect of naloxone (3×10^{-7} M), on the depressant effects of procaine on the extracellularly recorded compound action potential of frog's sartorius muscle strips. Means \pm S.E.M. ●, procaine alone; ○, procaine plus naloxone. $n = 3$. Although not indicated in the figure, the means with and without naloxone are not significantly different at $p = 0.05$. A paired t-test was conducted.

amplitude of the compound action potential. The addition of $3 \times 10^{-7}\text{M}$ naloxone, neither augmented nor antagonized the effects produced by procaine. Similar results were obtained for the conduction velocity (Figure 32).

Similar experiments were conducted using a low antagonistic concentration of naltrexone. As shown in Figure 33(a) and (b), naltrexone (10^{-7}M) did not antagonize the depression in the conduction velocity the threshold current or the maximum amplitude of the compound action potential, produced by procaine HCl.

(h) Dextromethorphan - HBr

When sartorius muscle bundles were exposed to dextromethorphan concentrations of 10^{-6}M or higher the maximum amplitude of the compound action potential was depressed. The dose-response curve obtained with 60 minute exposures to dextromethorphan is shown in Figure 34.

The effects of 3 dextromethorphan concentrations (Figure 35) were tested by themselves and in the presence of naloxone. Dextromethorphan in increasing concentrations progressively increased the threshold current and decreased the maximum amplitude of the compound action potential respectively. Naloxone (10^{-7}M) could not antagonize the depressant effects of dextromethorphan on either the threshold current or the maximum amplitude of the compound action potential.

(i) Tetrodotoxin

The effects of various concentrations of tetrodotoxin on the maximum amplitude of the compound action potential are shown in Figure 36. Concentrations of tetrodotoxin, $5 \times 10^{-9}\text{M}$ or higher produced a

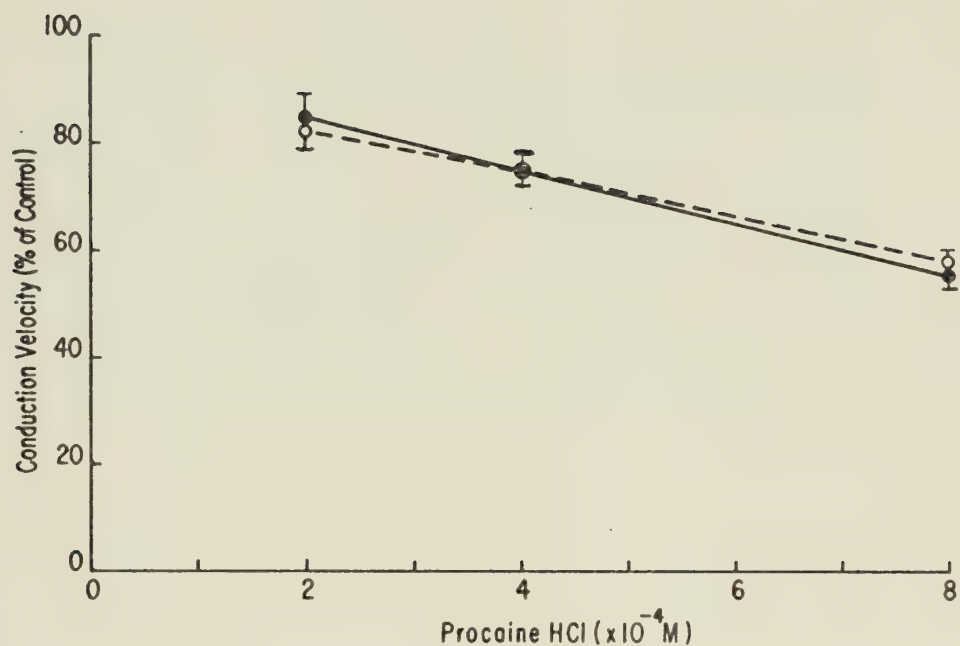
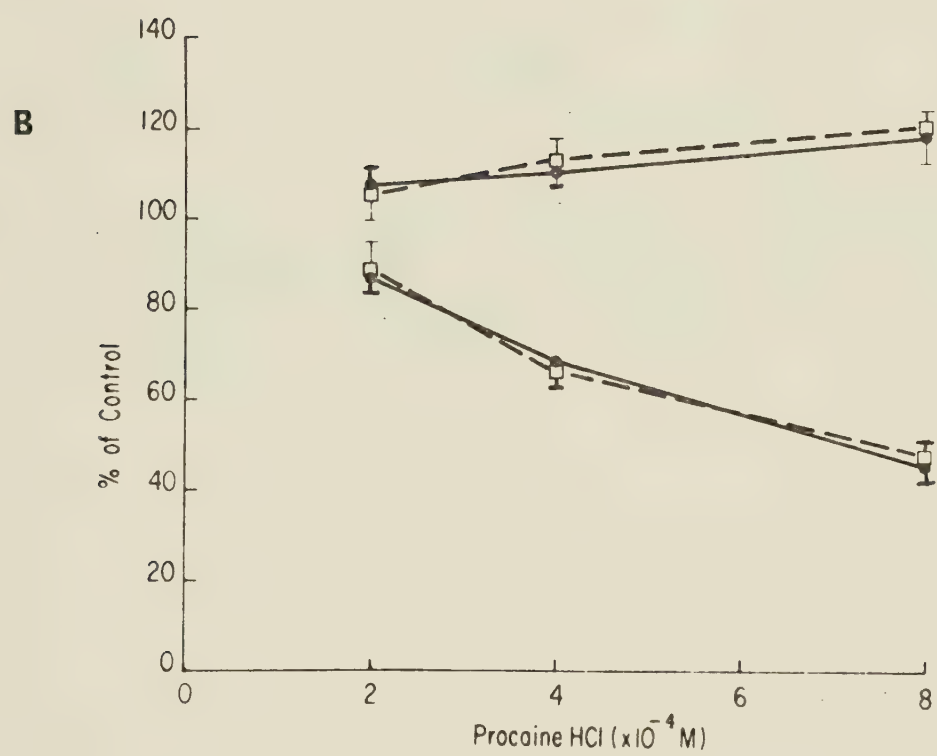
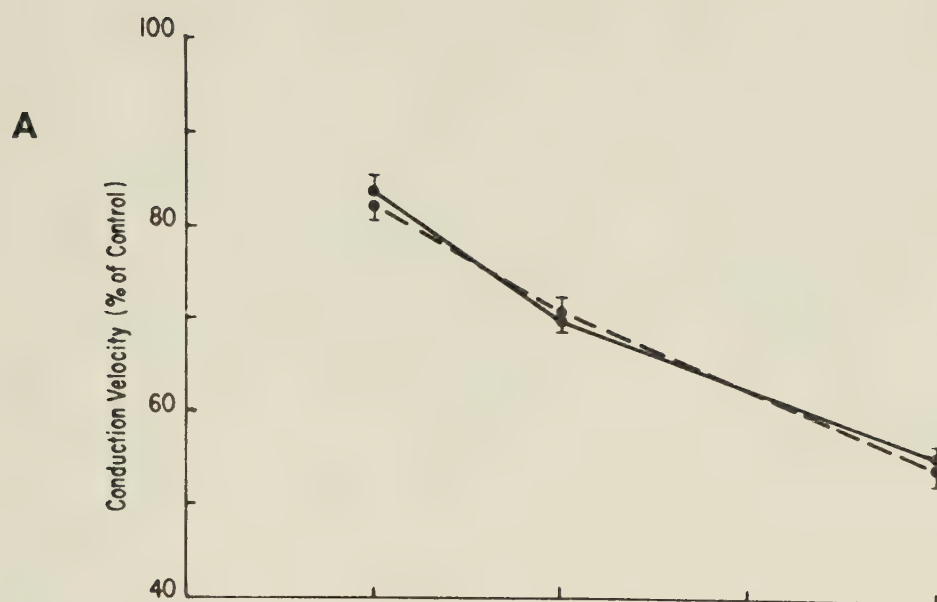


Figure 32: Lack of an antagonistic effect of naloxone (3×10^{-7} M), on the depressant effects of procaine on the conduction velocity of frog's sartorius muscle strips. Means \pm S.E.M. ●, procaine alone; ○, procaine plus naloxone. $n = 3$. All 3 muscles exposed to the 6 drug combinations. Although not indicated in the figure, the means with and without naloxone are not significantly different at $p = 0.05$. A paired t-test was conducted.

Figure 33: Lack of an antagonistic effect of naltrexone ($10^{-7}M$) on the depressant effects of procaine on the extracellularly recorded action potential of frog's sartorius muscle strips. In A, conduction velocity, mean \pm S.E.M.: ●---●, Procaine (Pro) alone; ●—●, Pro + Naltrexone (N). In B, Mean \pm S.E.M.: upper lines, threshold current; lower lines action potential maximum amplitude; ●—●, Pro only, □---□, Pro + N. Results from experiments with 6 muscle strips all exposed to the 6 drug combinations. In no case did the addition of Naltrexone significantly change the mean at $p = 0.05$. A paired t-test was conducted.



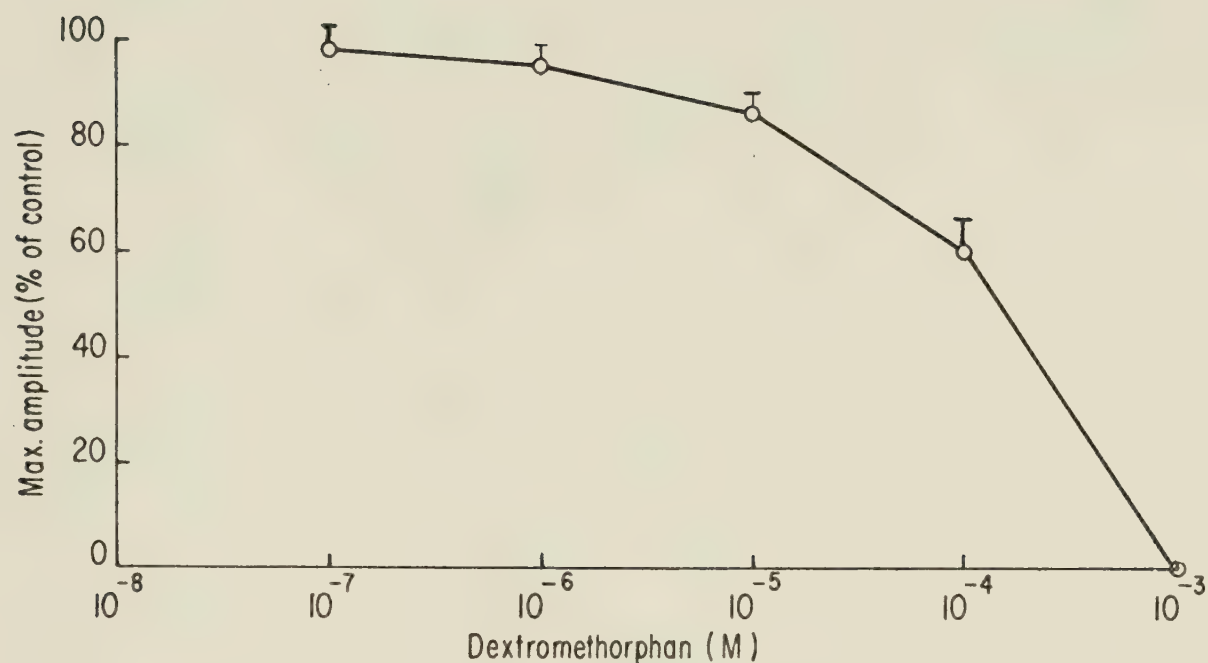


Figure 34: Effects of various concentrations of dextromethorphan on the maximum size of the compound action potential of frog sartorius muscle strips. Each mean and standard error was calculated from the mean responses of each of 3 preparations.

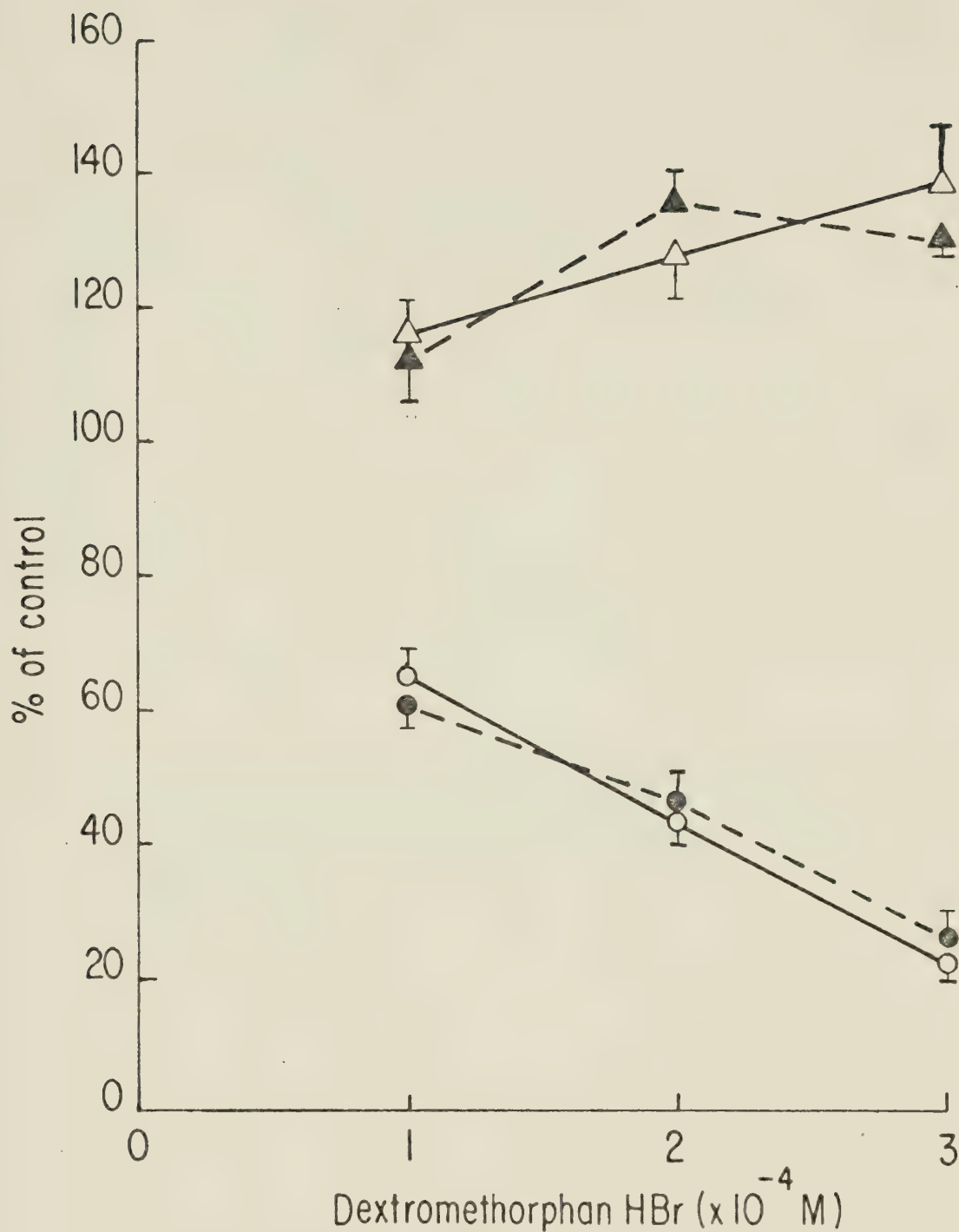


Figure 35: Lack of an antagonistic effect of naloxone ($10^{-7}M$) on the depressant effects of dextromethorphan (DMP) on the extracellularly recorded compound action potential of frog's sartorius muscle strips. Means \pm S.E.M. Threshold current: \triangle , DMP alone; \blacktriangle , DMP plus naloxone. Compound action potential: \circ , DMP alone; \bullet , DMP plus naloxone. $n = 3$. All muscles exposed to the 6 drug combinations. A paired t-test was conducted.

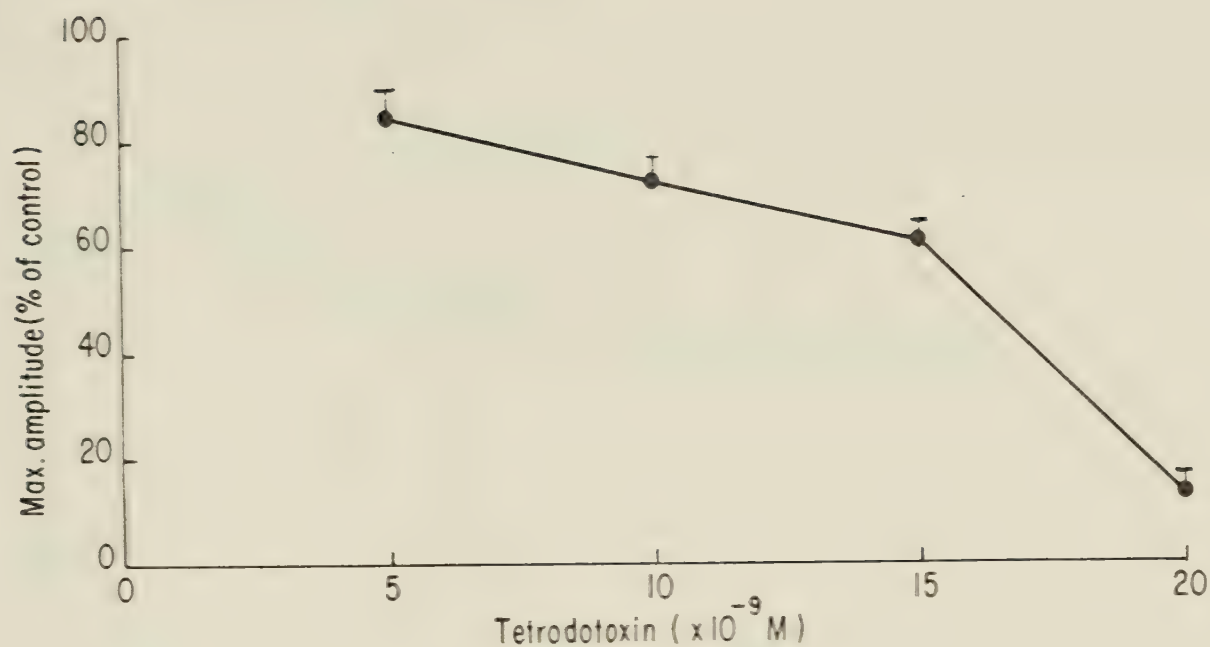


Figure 36: Effects of various concentrations of tetrodotoxin on the maximum size of the compound action potential of frog sartorius muscle strips. Each mean and standard error was calculated from the mean responses of each of three preparations.

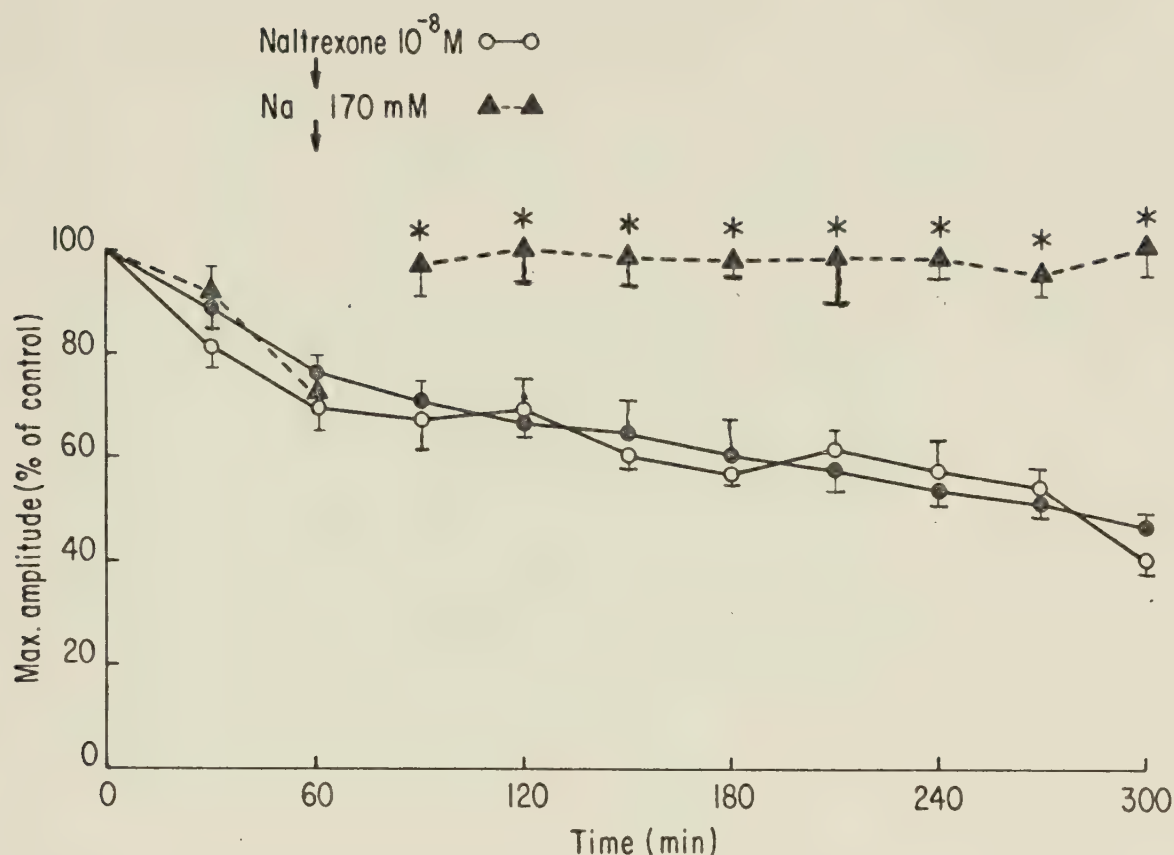
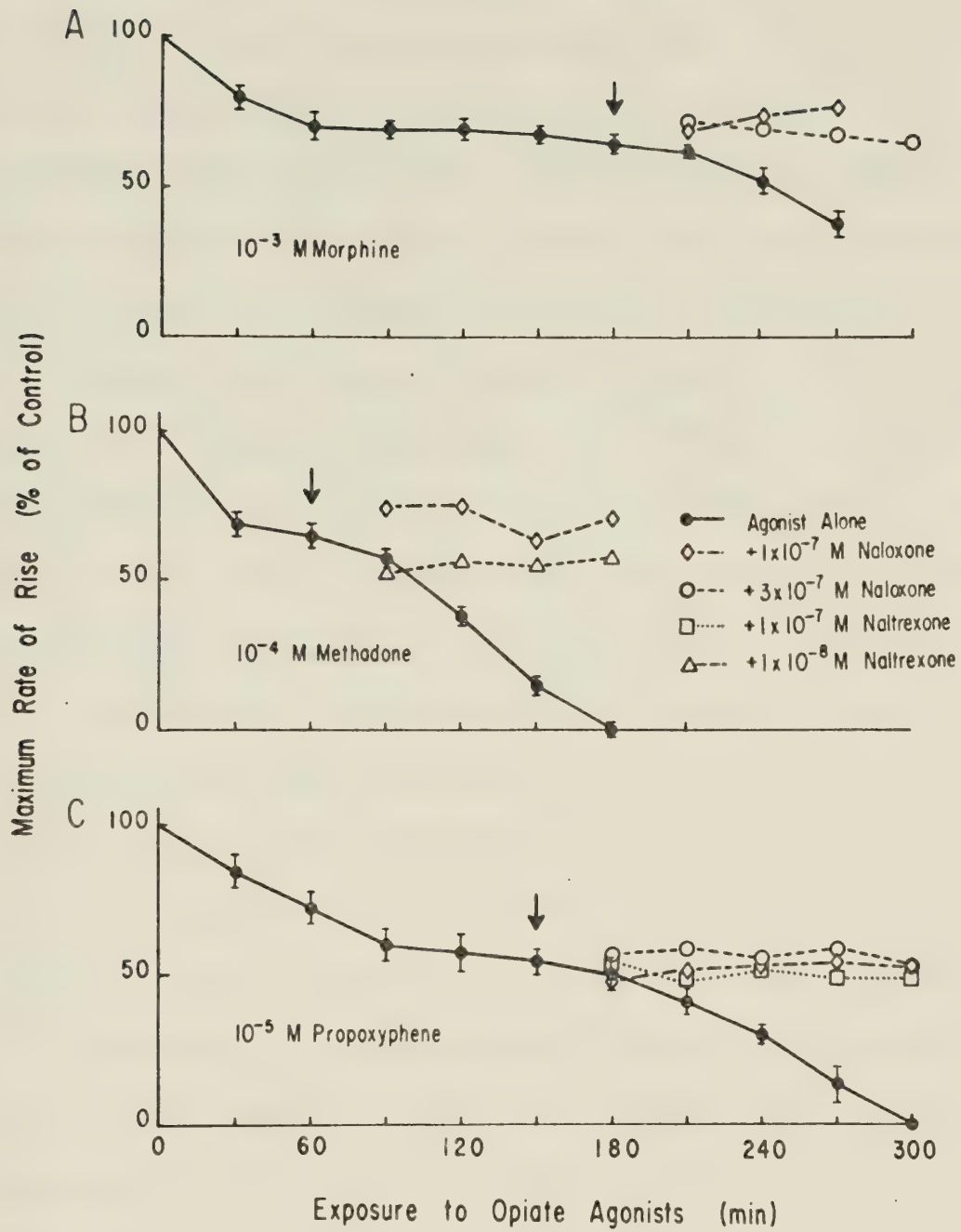


Figure 37: Effect of a relatively low tetrodotoxin (TTX) concentration (7.5×10^{-9} M) on the maximum amplitude of the compound action potential of the frog's sartorius muscles and the antagonistic effect of Na (170 mM) and Naltrexone (10^{-8} M) respectively. Results from experiments with 9 muscles; means \pm S.E.M.: 3 with TTX alone (●), 3 with TTX plus Na (▲) and 3 with TTX plus naltrexone. Na or naltrexone added between 60-90 minutes. *, means with and without Na significantly different at $p = 0.05$. An unpaired t-test was conducted.

Figure 38: Antagonism by naloxone or naltrexone of the second phase of the biphasic depression of the action potential maximum rate of rise in frog sartorius muscle fibres produced by some opiate agonists. ●, agonist alone (10^{-3} M morphine in A; 10^{-4} M methadone in B; and 10^{-5} M propoxyphene in C); ◇, agonist + 10^{-7} M naloxone; o, agonist + 3×10^{-7} M naloxone; □, agonist + 10^{-7} M naltrexone; △, agonist + 10^{-8} M naltrexone. Agonist added to the solution bathing the muscle at time 0 and antagonist added immediately following the recording period indicated by the arrows. The number of muscles (n) for the agonist alone: 8 in A; 7 in B to 60 minutes and 3 thereafter; and 9 in C. In A: ◇ and o, two muscles each; in B: ◇ and △, 2 muscles each; and in C: o and □, 1 muscle each; and ◇, 2 muscles.



progressive decline in the amplitude of the action potential.

In another series of experiments muscle bundles were continuously exposed to a low concentration of tetrodotoxin (7.5×10^{-9} M) either with naltrexone (10^{-8} M) or excess sodium (170 mM) or without any of the latter two drugs. The results obtained with exposures up to 300 minutes are presented in Figure 37. In all tests, after the initial control responses were obtained, the muscles were placed in a solution with only tetrodotoxin at time 0. In 5 of the experiments the muscles remained in this solution except when testing the electrical response. In the rest of the experiments the muscles were placed in a solution containing the same tetrodotoxin concentration plus either sodium or naltrexone at time 60 minutes and remained in this solution.

With this tetrodotoxin concentration (7.5×10^{-9} M) there was a progressive decrease in the maximum amplitude of the compound action potential. Naltrexone (10^{-8} M) when added at 60 minutes did not antagonize this decrease in the amplitude of the action potential. Sodium (170 mM) added at 60 minutes significantly antagonized the depression in the compound action potential amplitude.

Concentrations Required to Produce 50% Change

The concentrations of some drugs required to produce a 50% change are shown in Table 2.

Intracellular Studies

(a) Morphine, Methadone, Propoxyphene and Sodium Conductance

Several experiments were conducted using 10^{-3} M morphine (Figure 38a); 10^{-4} M methadone (Figure 38b); 10^{-5} M propoxyphene (Figure 38c); either with or without the addition of naloxone or naltrexone. The effects produced on the maximum rate of rise of the action potential

by these drugs are summarized graphically in Figure 38. With each of the three opiate agonists used in these experiments a concentration was found which produced a biphasic decline in the action potential maximum rate of rise (Figure 38). When naloxone or naltrexone was added at the points indicated by the arrows, its effect was to antagonize only the second phase of the agonist depression (Figure 38), indicating a fundamental difference in the mechanisms producing the two phases. The opiate agonists differed only in the concentrations required to demonstrate this biphasic decline and in the time course of their effects.

(b) Naloxone

The effects of a single low antagonistic concentration of naloxone ($3 \times 10^{-7}\text{M}$) on the maximum rate of rise were investigated. As shown in Figure 39, naloxone at a concentration of $3 \times 10^{-7}\text{M}$ produced a progressive decrease in the maximum rate of rise.

Figure 40 shows the effect produced by a high concentration (10^{-3}M) of naloxone on the action potential maximum rate of rise. With increased exposure time the maximum rate of rise was increasingly depressed. In contrast to the effects observed with opiate agonists, the depression in the maximum rate of rise appeared to be monophasic. When a low concentration (10^{-7}M) of naltrexone was present in the bathing medium with the naloxone at the start of the experiments, naltrexone significantly reduced the depression in the maximum rate of rise.

(c) Naltrexone

Figure 41 shows the effects of a single low antagonistic concentration of naltrexone ($3 \times 10^{-8}\text{M}$) on the action potential maximum rate

Table 2 The concentrations of some drugs required to produce a 50% decrease in the conduction velocity (Cond Vel) and maximum amplitude (Max Ampl) and a 50% increase in the threshold current (Thres Cur) for extracellularly recorded, compound action potentials in frog's sartorius muscle strips. n, number of muscle strips tested.

<u>CONCENTRATION (M) REQUIRED TO PRODUCE 50% CHANGE</u>				
<u>Drug</u>	<u>Max Ampl</u>	<u>Cond Vel</u>	<u>Thres Cur</u>	<u>n</u>
Morphine HCl	$4.3 \pm 0.9 \times 10^{-3}$	$3.8 \pm 0.9 \times 10^{-3}$	$1.6 \pm 0.7 \times 10^{-3}$	6
Methadone HCl	$>6.0 \times 10^{-5}$	$5.6 \pm 1.0 \times 10^{-5}$	$3.6 \pm 0.8 \times 10^{-5}$	6
Propoxyphene HCl	$4.7 \pm 0.6 \times 10^{-5}$	$4.1 \pm 0.8 \times 10^{-5}$	$2.2 \pm 0.6 \times 10^{-5}$	5
Meperidine HCl (from Frank, 196)	$4.6 \pm \times 10^{-4}$	—	—	5
Dextromethorphan	$>1.0 \times 10^{-4}$	$>1.0 \times 10^{-4}$	$0.5 \pm 0.4 \times 10^{-4}$	3
Naloxone	$7.5 \pm 1.7 \times 10^{-3}$	$2.3 \pm 0.2 \times 10^{-3}$	$1.5 \pm 0.1 \times 10^{-3}$	3
Naltrexone	$>1.0 \times 10^{-3}$	$8.7 \pm 0.6 \times 10^{-4}$	$>1.0 \times 10^{-3}$	6
Procaine HCl	$4.8 \pm 1.9 \times 10^{-4}$	$6.3 \pm 1.4 \times 10^{-4}$	$5.8 \pm 1.3 \times 10^{-4}$	6
Tetrodotoxin	$1.6 \pm 0.4 \times 10^{-8}$	$>2.0 \times 10^{-8}$	$>1.9 \times 10^{-8}$	3

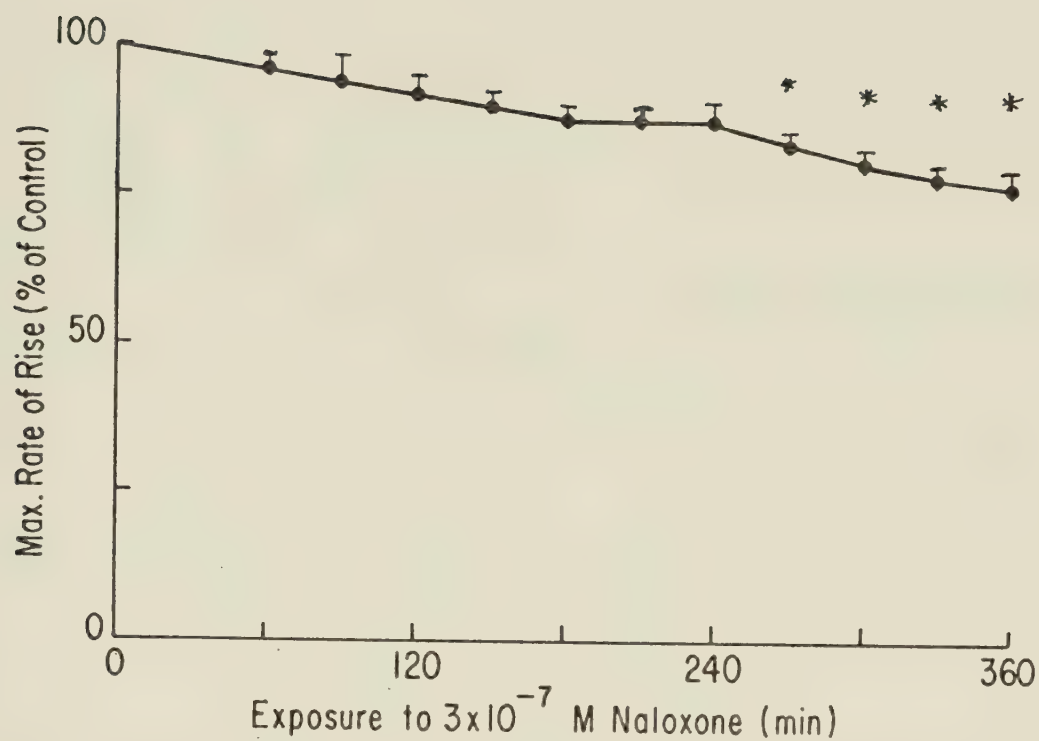


Figure 39: Effects of a single low antagonistic concentration (3×10^{-7} M) of naloxone on the action potential maximum rate of rise of frog's sartorius muscle fibres. Means \pm S.E.M. Curves plotted as described in Figure 8. $n = 3$.

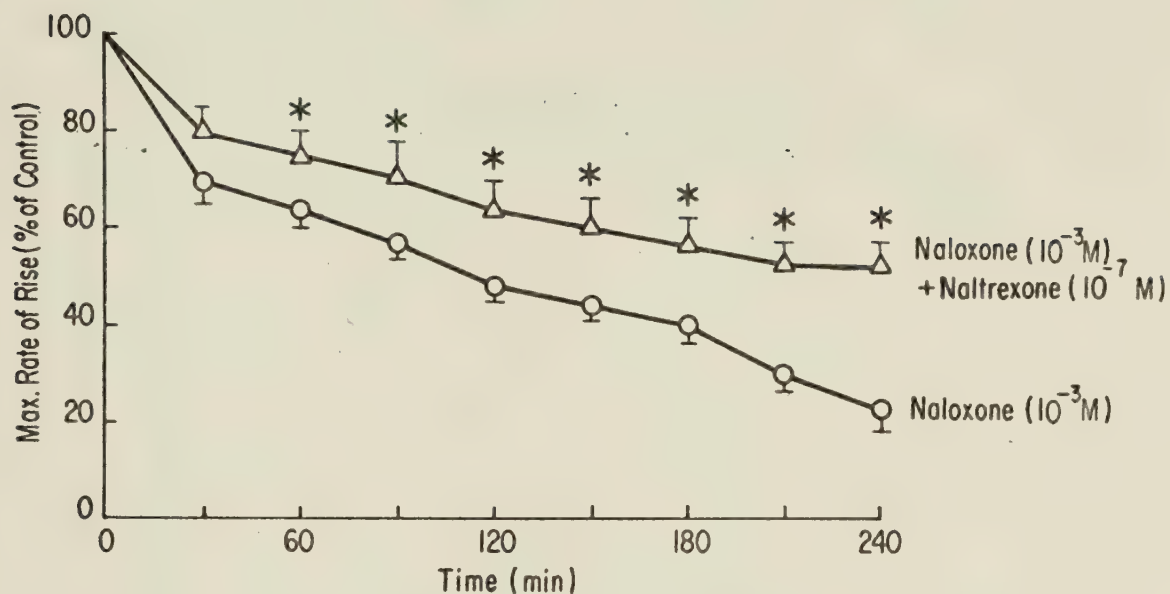


Figure 40: Antagonistic effect of a low concentration ($10^{-7}M$) of naltrexone on the decrease in the intracellularly recorded action potential maximum rate of rise produced by a high concentration ($10^{-3}M$) of naloxone. Means \pm S.E.M. Results from experiments with 6 muscles, 3 exposed to naloxone alone (\circ), and 3 exposed to naloxone plus naltrexone (Δ). *, means with and without naltrexone, significantly different at $p = 0.05$. An unpaired t-test was conducted.

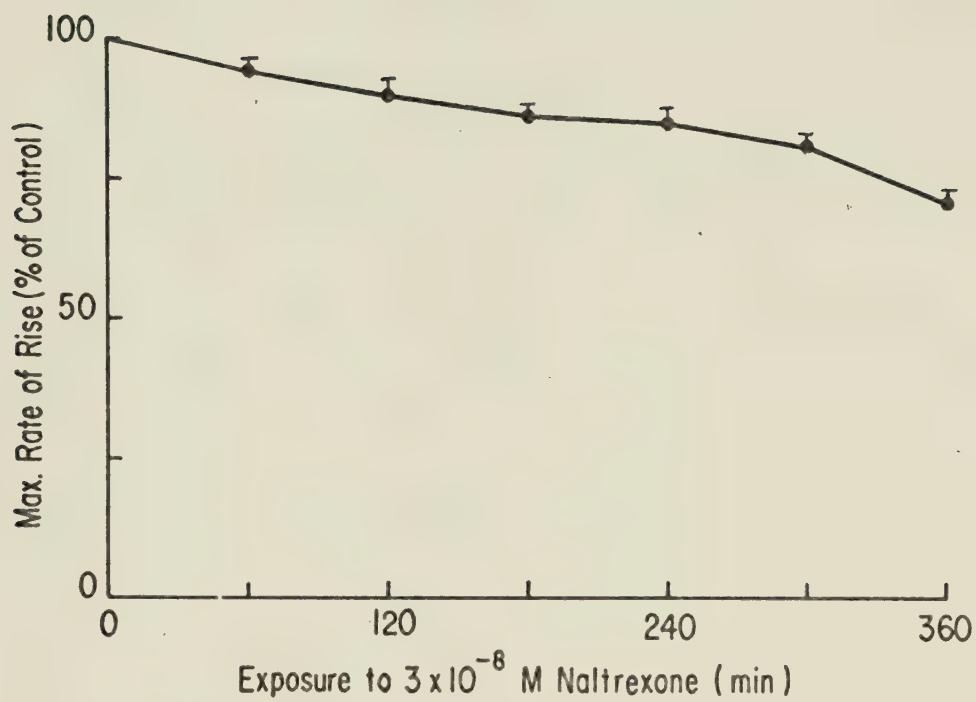


Figure 41: Effects of a single low antagonistic concentration of naltrexone (3×10^{-8} M) on the action potential maximum rate of rise of frog's sartorius muscle fibres. $n = 3$, means \pm S.E.M. Results obtained and plotted as in Figure 8.

of rise. As can be seen from Figure 41, there was a progressive decline in the maximum rate of rise of the action potential, on exposure to naltrexone ($3 \times 10^{-8}\text{M}$).

In another type of experiment muscles were continuously exposed to a single concentration of naltrexone ($5 \times 10^{-4}\text{M}$) either with or without naloxone (10^{-7}M). The results obtained with exposures to 240 minutes are presented in Figure 42. With this naltrexone concentration ($5 \times 10^{-4}\text{M}$) there was a progressive and a monophasic decrease in the maximum rate of rise. In experiments in which naloxone (10^{-7}M) was present also, the decrease in the maximum rate of rise due to naltrexone could not be antagonized or be prevented.

Extracellular Sodium and Sodium Conductance

Figure 43 shows the effect of extracellular sodium concentration on the depressant effects of various drugs on the maximum rate of rise of the action potential. In every case in Figure 43 the drug appears to depress the maximum rate of rise, this effect becoming more pronounced with decreasing sodium concentrations. Also in every case in Figure 43 increasing the extracellular sodium appears to antagonize the depressant effect of the drug on the maximum rate of rise, again this effect becomes more pronounced with increasing extracellular sodium concentration. There also appears to be a linear relationship between the extracellular sodium concentration and the maximum rate of rise, of the action potential.

Effects on the Potassium Conductance

It has been shown that there is a linear relation between the action potential size and the action potential maximum rate of fall (56). When a drug reduced \bar{g}_K it displaces this linear relation to

higher action potential values without significantly changing the slope of the line. An effect of this type produced by methadone is illustrated in Figure 44. Antagonistic concentrations of naloxone ($10^{-7}M$) did not appear to have any effect on the depression in $\bar{g}K$ produced by methadone.

In order to compare the effects produced by the various drugs and drug combinations, linear regression curves were calculated for each experiment and condition. From these curves a maximum rate of fall was determined at a standard action potential size of 130 mV. The values thus obtained were used to calculate the change in inward current (ΔI_i). Results obtained in the present study are listed in Table 1. The three agonists, employed in the concentrations required to produce a biphasic decline in $\bar{g}Na$, produced about the same reduction in $\bar{g}K$ (35-37%).

The addition of antagonistic concentrations of naloxone or naltrexone produced inconsistent changes in $\bar{g}K$. Thus $\bar{g}K$ was further reduced when the antagonist was added to methadone, it was increased when the antagonist was added to propoxyphene, and it was unchanged in the tests with morphine.

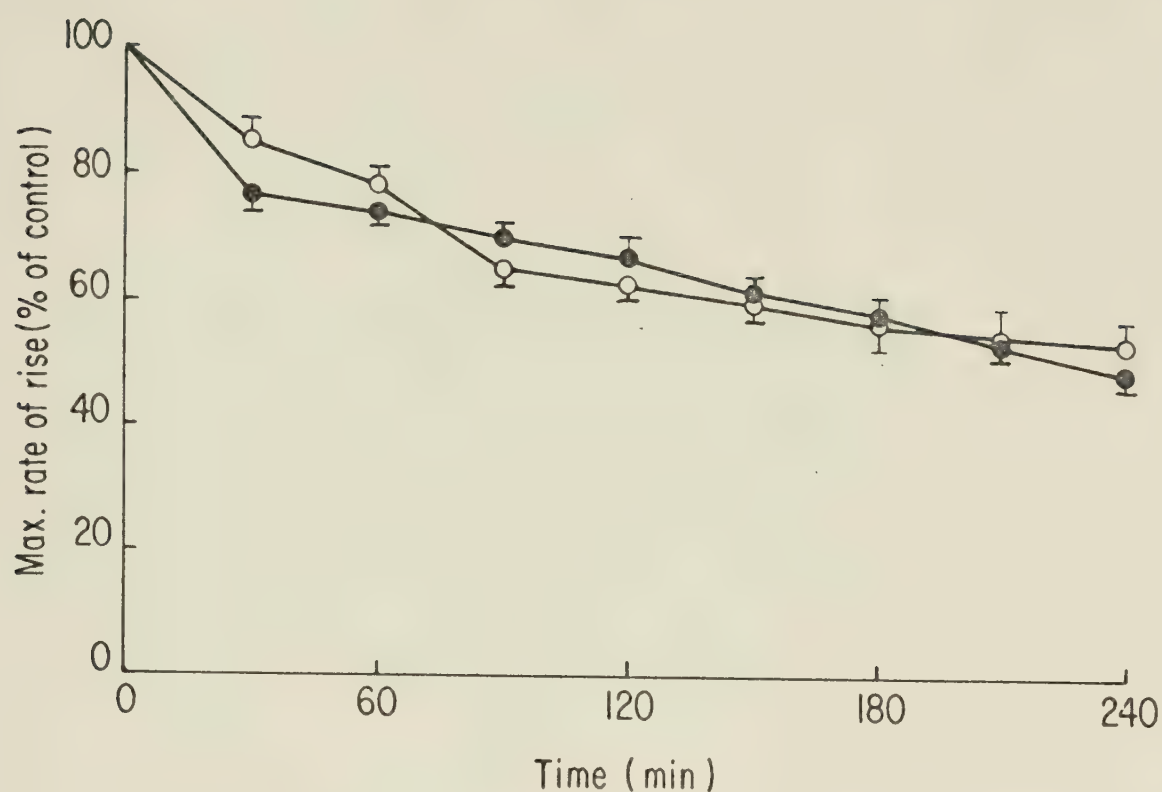
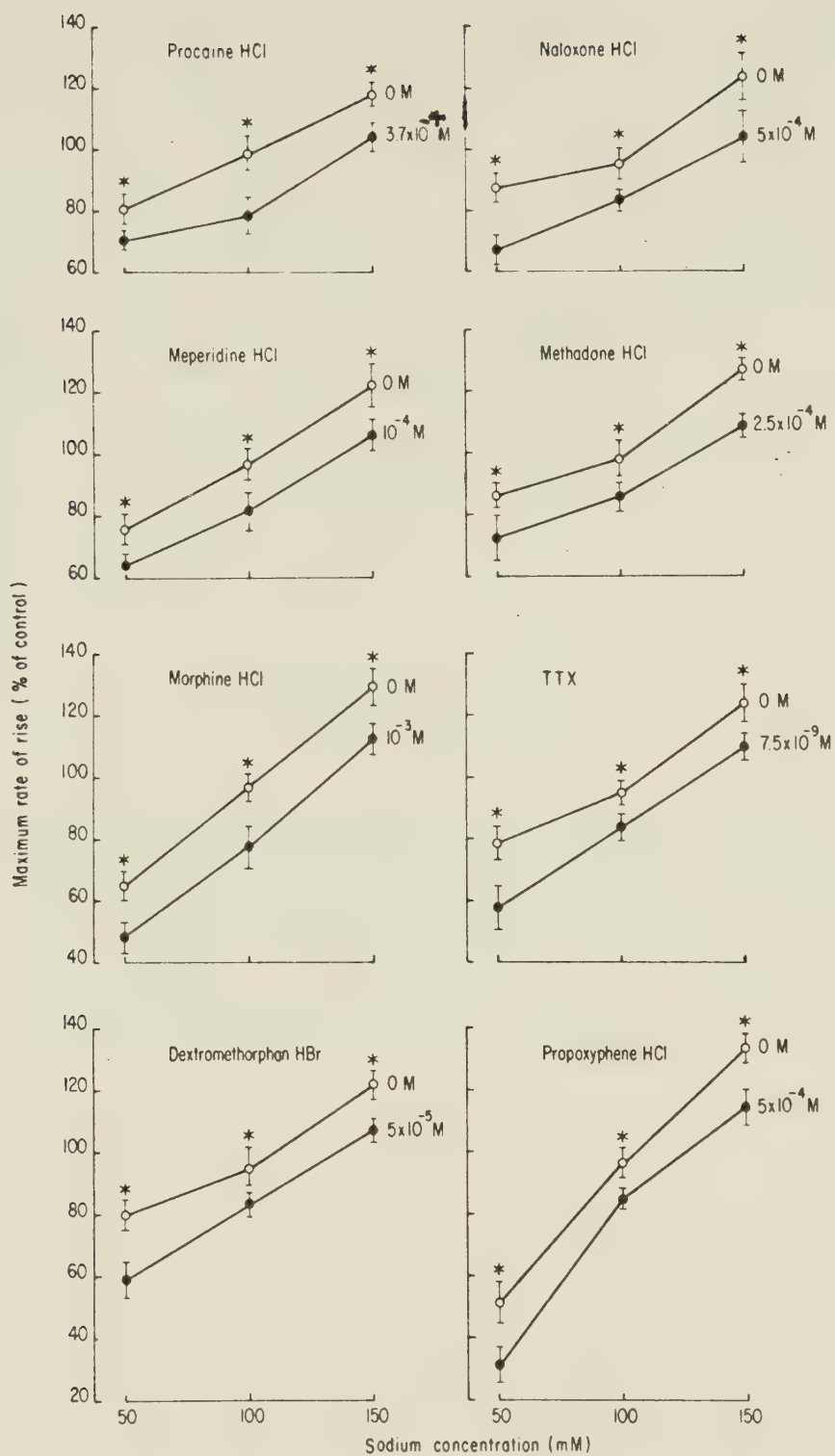


Figure 42: Lack of an antagonistic effect of naloxone ($10^{-7}M$) on the depressant effects of naltrexone ($5 \times 10^{-4}M$) on the action potential maximum rate of rise. Means \pm S.E.M. ●, naltrexone alone ($n = 3$); o, naltrexone plus naloxone ($n = 3$). An unpaired t-test was conducted.

Figure 43: Effect of extracellular sodium concentration and of various CNS depressant drugs on the maximum rate of rise of intracellularly recorded action potentials from the frog's sartorius muscle fibres. In each case, upper curve, Ringer's solution without drug; lower curve, with drug. Drug concentrations indicated on the right for each graph. In every case, mean and standard error derived from the mean values obtained in each of 3 separate preparations. The maximum rate of rise is expressed as a percentage of control for all graphs. A paired t-test was conducted.



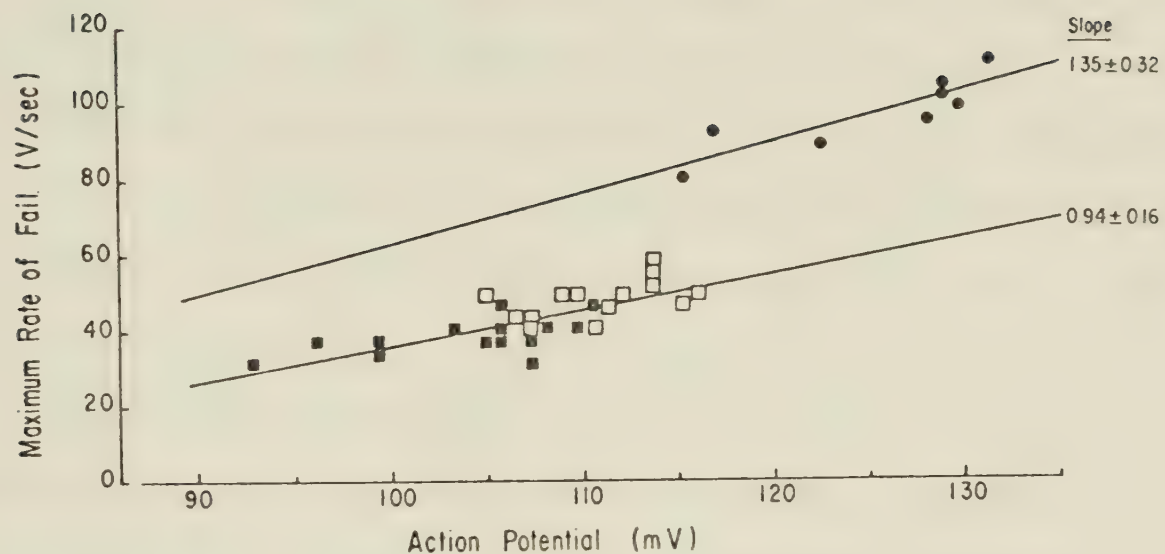


Figure 44: Methadone and methadone plus naloxone effects on the action potential maximum rate of fall. Methadone, 10^{-4}M placed in the solution bathing the muscle at time 0 and naloxone, 10^{-7}M at 65 minutes. ●, pre-drug controls; □, 25-35 minutes and 55-65 minutes; ■, 145-155 minutes and 175-185 minutes. Slopes significantly different from 0 at $p = 0.05$.

VI. DISCUSSION

Direct electrophysiological investigations of the electrical properties of the in vitro preparation have considerable advantages over in vivo studies, in that the modification of the electrophysiological measurements following treatment with depressant drugs, allows not only more precise comparisons of the potencies, efficacies and affinities of a variety of different compounds, but can also help to elucidate the mechanism involved in the excitability block at the cellular level.

Studies with extracellular electrodes showed that increasing concentrations of propoxyphene, methadone, morphine, meperidine, dextromethorphan, naloxone, naltrexone, tetrodotoxin and procaine, caused a progressive depression of the maximum amplitude of the compound action potential and the excitability of the muscle fibres. Intracellular recordings from individual cells showed that all these drugs produced a depression in the membrane excitability, as indicated by the decrease in the maximum rate of rise. The resting membrane potential, however, remained unchanged.

The increase in membrane sodium conductance which follows an adequate depolarization of the fibre membrane is a major factor in determining the rate of rise of the action potential and the maximum amplitude of the action potential (10, 28). This is consistent with the two relationships pointed out by Hodgkin and Katz (219, equations 3 & 8.1) and thus the assumption has been made here that the maximum rate of rise of the action potential is proportional to the sodium permeability of the membrane during the rising phase of the action potential.

The results discussed above, interpreted in terms of the ionic hypothesis, suggest that the sodium conductance in the active membrane is reduced in the drug treated muscles. Thus, the suppression of the excitation and the amplitude of the action potential by the drugs tested appears to be related to a depression of the sodium ion permeability of the active membrane of the muscle fibre. According to the "sodium hypothesis" in a sodium-deficient medium the depressant action of the anesthetics would be intensified, whereas such an effect should be antagonized by raising the concentration of sodium ions in the bathing medium. In the present investigation it has been shown that addition of excess sodium (170 mM) into the extracellular medium antagonized the depressant action of all the drugs tested on the rate of rise of the action potential (used as a measure for sodium inward current). These findings not only lend further support to the mechanism of action (i.e. sodium depressant effect) proposed here for the drugs used in this study, but also substantiate the proposals made previously by some investigators for general CNS depressant drugs (44, 45, 46, 195). Nevertheless all have had to contend with the fact that in addition to producing this common pattern of general CNS depression, these drugs also produce other effects on the CNS which are unique to themselves or to only a few drugs with closely related structures. Thus even though a reduction in membrane permeability may account for the ability of anesthetics to block excitability, the exact mechanism by which the permeability is affected remains in many cases unknown.

Consistent with the above discussion, are the findings of this study which show that the drugs investigated appear to depress \bar{g}_{Na}

in the active membrane through a variety of mechanisms. Thus drugs such as propoxyphene, methadone, morphine and meperidine appear to depress \bar{g}_{Na} via two mechanisms. Dextromethorphan, naltrexone, procaine and tetrodotoxin all decrease by a mechanism not involving opiate receptors. However, it is well established that tetrodotoxin blocks sodium channels from the external aspect of plasma membranes (378) by an effect on a specific drug receptor and that procaine decreases the sodium conductance by acting at the inner aspect of the plasma membrane (30, 31). Further local anesthetics in addition to their above site of action also expand membranes and increase membrane fluidity (42, 44, 58). Thus despite the fact that the final action of tetrodotoxin and procaine appear to be identical, the respective mechanisms of action of these two drugs obviously differ. Similar arguments hold for the other drugs tested. Naloxone in high concentrations decreases \bar{g}_{Na} and this depression in excitability can be antagonized by naltrexone. This indicates that naloxone is not a "pure" antagonist but rather a partial agonist because it can have at different concentrations, either an agonist (stimulating), or an antagonist effect by acting on a single type of drug receptor.

In addition in the present study it was shown that the depressant effects produced by procaine, tetrodotoxin and dextromethorphan can not be antagonized by low concentrations of opiate antagonists. Further dextromethorphan in low concentrations does not antagonize the depressant effects of opiate agonists. This indicates that opiate antagonists do not produce a non-specific antagonistic effect to all excitability depressant drugs. These results are consistent with the view that there are opiate drug "receptors", on sartorius muscle fibre

membranes and drug activation of these receptors inhibits action potential production.

The drugs studied in this investigation could cause reduction of the sodium current by one or several of the following: (a) Membrane Expansion, (b) Membrane Fluidization, (c) Receptor-Mediated Process, and (d) Displacement of Membrane Calcium. Most likely all these effects occur simultaneously, and one or the other of these mechanisms may dominate in a particular system.

Many investigators (55, 66, 68, 69, 74, 75, 76, 77, 78, 79, 80, 82, 97, 98, 101, 116, 118, 155, 164, 168, 172, 182, 193, 194, 195, 196) have demonstrated that opiates exert their effects via interaction with opiate receptors. This investigation has furnished similar evidence. Extracellular studies showed that propoxyphene, methadone, morphine and meperidine produced excitability depressions that can be antagonized by low concentrations of naloxone or naltrexone. It was also shown that antagonistic concentrations of naltrexone can antagonize the agonistic effects of high concentrations of naloxone but that antagonistic concentrations of naloxone can not antagonize the agonistic effects of high concentrations of naltrexone. These results indicate that naloxone is a drug with both agonist and antagonist effects whereas naltrexone appears to have only an antagonistic effect on the 'opiate receptor' in frog's sartorius muscle membrane.

Studies with intracellular microelectrodes indicated that morphine, methadone and propoxyphene block action potential production by two mechanisms, (i) a non-specific mechanism in which the increase in sodium conductance (\bar{g}_{Na}) and in potassium conductance (\bar{g}_K) are

depressed and, (ii) an opiate drug receptor-mediated mechanism, causing a specific depression of \bar{g}_{Na} . Low, antagonistic concentrations of opiate antagonists antagonize only the effects produced by the second mechanism (ii). These results confirm the pattern of biphasic block first proposed for meperidine by Frank (56). These findings also confirm the previous contention (56, 194, 258, 259) that opiates produce their actions by both specific and non-specific effects. A possibility arises that if the muscles had been previously preincubated with a low concentration ($\sim 10^{-7}M$) of opiate antagonists that it would have been also possible to antagonize the initial phase of the biphasic depression, on addition of the agonists. Several such experiments were conducted by Frank (personal communication) for meperidine ($3.5 \times 10^{-4} M$) and naloxone ($3 \times 10^{-7} M$). Frank found that the decreases in the maximum rate of rise induced by meperidine were similar whether the muscles were preincubated by naloxone or not. As shown in Figure 39, even at antagonist doses naloxone depresses the maximum rate of rise and preincubating with naloxone would have further confused the issue. Further as shown in Figures 21 and 38, the antagonistic effect of opiate antagonists does not become apparent until 90 minutes (Figure 21) and 30 minutes (Figure 38) after the antagonists are added. This antagonism occurs at a time when the second phase of the biphasic depression in the maximum rate of rise is developing and is consistent with the conclusion that the antagonists only antagonize the receptor mediated depression (phase II of the biphasic depression).

In contrast to the biphasic depression produced by opiate ago-

nists it was found that high concentrations of naloxone (10^{-3}M) or naltrexone ($\sim 10^{-4}\text{M}$) inhibit the maximum rate of rise in a monophasic fashion. Only the effects of naloxone can be antagonized by concomitantly employing low concentrations of the other opiate antagonist. This confirms the results of the extracellular technique and gives support to the possibility that naloxone is a partial agonist. Such a possibility might coincide with the findings of other investigators (383). The definition of a partial agonist according to Ariens theory of "competitive dualism in action" is that such compounds possess intermediate intrinsic activity. On addition of a partial agonist to an organ which is in contact with a dose of a related compound, with a higher intrinsic activity, the partial agonist should competitively displace the full agonist from the receptors. When the partial agonist is added in a high enough concentration all receptors will be occupied by it and the effect becomes equal to the maximum effect of the partial agonist.

However our results with naloxone are at variance with this definition. Naloxone in low doses ($\sim 10^{-7}\text{M}$) antagonized opiate agonist effects, yet at high doses acted as an agonist. Further increases in the concentration of naloxone served to increase its agonistic effects. At high concentrations ($\sim 10^{-3}\text{M}$) the agonistic effects of naloxone paralleled those of opiate agonists. Earlier studies by Frank (196) and Frazier et al. (194, 359) had shown that the effects of opiate antagonists in high concentrations added on to the effects of opiate agonists. The results of this thesis and those of Frank and Frazier et al. are clearly in disagreement with the above theory of Ariens.

These results are more in agreement with Martin's theory of Receptor Dualism (155). This theory states that there are two "analgesic" receptors, one where full agonists act and partial agonists act as competitive antagonists and the other where agonists are inactive and partial agonists are agonists. Although these receptors are distinguishable they must have very similar stereochemical configurations.

As stated earlier opiate agonists produce a biphasic decline in the maximum rate of rise of the action potential in frog sartorius muscle. In this study, of the two inhibitory effects on \bar{g}_{Na} produced by opiate agonists only the second phase can be antagonized by low concentrations of opiate antagonists. Thus during the initial phase opiate agonists produce a non-specific local anestheticlike effect on the active electric characteristics of the membrane. An obvious explanation for the time course of the second phase is that the receptor sites for this mechanism are located on the inner surface of the plasma membrane and that the agonist molecules attached to these receptor sites are in equilibrium with the drug concentration in the myoplasm. Thus the proportion of receptor sites occupied by agonist molecules would be determined by the sarcoplasmic concentration of the opiate agonists. There would be no effect produced by this mechanism until a 'threshold' sarcoplasmic concentration is reached. Characteristically, drug dose-response curves are S-shaped and so even after the threshold concentration is obtained, only a small effect would be produced until the steep portion of the dose-response curve is reached. Thereafter there would be a large increase in effect for each small increase in sarcoplasmic drug con-

centration.

In different studies, on exposure of the muscles to single "low antagonistic" concentrations of naloxone or naltrexone, the antagonistic effect changes to an agonistic effect. This presumably is due to the fact that the intracellular concentration of these drugs is "building up" during this time. This also suggests that the opiate receptors are located on the intracellular surface of the muscle membrane. Intracellular locations of opiate receptors have also been proposed by other investigators (56, 193, 194, 384).

However, alternative explanations for the location of the opiate 'receptor' in this study are possible. Thus it is conceivable that the biphasic pattern observed in the present studies is due to the migration of receptors from intracellular to extracellular loci. Alternatively, the biphasic pattern may be due to conformational changes or increased cooperativity that could be developing between "receptor protomers". Some workers (76) have indicated that the opiate receptor should be extracellular in order to be accessible to the endogenous ligands, which are known to be peptides of relatively large size and therefore not expected to pass through cell membranes, unless there is a specialized uptake system. Supporting this conclusion are the results of Hitzeman and Loh (140), who showed that after tryptic digestion of nerve ending particles, the stereospecific opiate binding is eliminated. This indicates that the stereospecific opiate binding site is probably located on the external surface of the nerve ending particles.

This dichotomy regarding the cellular distribution of opiate receptors may be resolved by proposing that the opiate receptor

could conceivably be a "transmembrane stud". However, it is more likely that the conflicting conclusions exist because of species or tissue differences. Further, the opiate receptors being studied by different investigators may not all be identical; i.e. there may exist a heterogeneous group of opiate receptors each mediating the different effects of opiates in different tissues under different conditions. Such an idea is currently being entertained by an ever increasing number of investigators (297, 298, 345, 346, 347, 348, 386).

The fact that opiates mediate their effects by interaction with opiate receptors does not invalidate the concept that CNS depressants act by inhibiting mainly the sodium conductance because this inhibition could occur as a consequence of or subsequent to the interaction of opiates with their "receptors". The receptors may be closely associated with or be part of the ionic channels. The precise relationships between these receptors and the ionic channels remains elusive at the present time. It is possible that opiates induce a conformational change in the receptors and this in some way effects the "ion conductance modulator" and thus the permeating channel.

The common denominator between all the drugs studied in this investigation is that they all inhibit the Na^+ conductance in vitro, yet many of these drugs have different pharmacological profiles in vivo. It is possible that the different categories of drugs may reflect different membrane sites of attachment or action (some drugs may even act by several different mechanisms) resulting in different final pharmacological efficacies or intrinsic activities. For example, the opiates may act deep within the substance of the cell membrane

affecting the Na^+ channel relatively slowly or inefficiently, procaine may not only act deep inside the membrane but also act at the outer aspect and also within the membrane and tetrodotoxin conceivably attaches directly to the Na^+ channel and so has the highest efficacy. Supporting such a concept is the work of Craviso and Musacchio (141) who showed that the opiate receptor is not related to or part of the TTX or local anesthetic "receptors".

The results of this study indicate that there are opiate drug receptors located on the inner surface of the muscle membrane, associated with the 'sodium channels' and that drug activation of these receptors interferes with the opening of the 'sodium channels' normally produced by membrane depolarization.

A technical difficulty encountered is the lack of a clear cut distinction between the local anestheticlike and opiate receptor effects of the drugs studied. All opiate agonists studied exhibited these dual properties. This problem was further compounded by the finding that the local anestheticlike effect preceded the opiate receptor effect. If high doses of opiate agonists were employed the local anesthetic effect completely blocked excitability before the opiate receptor effect occurred. If low concentrations were employed the local anestheticlike and opiate receptor effects merged and both appeared to develop at the same rate. Also the time course of the latter study was greatly prolonged.

The findings of this thesis that there are opiate receptors on skeletal muscle contradict the reports of Pert et al. (118) who found that opiate receptors were confined only to nervous tissue. As

outlined in the introduction, binding studies have many drawbacks. For instance in many binding studies involving the brain, the cerebellum is routinely discarded because it possesses no binding sites (100). Yet studies with other techniques e.g. microiontophoresis, and local application have shown that the cerebellum does possess opiate receptors (352). Also, the amygdala has been shown to possess one of the highest density of binding sites in the brain, yet it is insensitive to local application of opiates (132). Further a recent report (386) has shown that enkephalins are present in the cerebellum of rats. Another group of investigators (387) has demonstrated that enkephalins act directly at or within smooth muscle cells. Such discrepancies cast serious doubts on the conclusions of some binding studies and provide caution to future investigators who may attempt to reach conclusions, with results derived from such studies.

The lack of correspondence between the concentrations of opiates employed in this study and those employed clinically could be due the existence of different groups of opiate receptors. Martin (346) and Lord et al. (348) have demonstrated the existence of heterogeneous opiate receptors on various tissues. The lack of correspondence could also be attributed to the differences in pharmacokinetics that exist in vivo and in vitro.

Finally it should be pointed out that the greater than 100% of maximum control compound action potential amplitude observed here in some of the extracellular experiments was probably an artifact of the bipolar extracellular recording system. Since the amplifier records the difference in the electrical potential occurring at the two

recording electrodes 2 mm apart, a decrease in conduction velocity will tend to increase the potential recorded and if simultaneously the amplitudes of the action potentials in the individual muscle fibres are at or near control values the maximum amplitude of the extracellularly recorded compound action potential would be greater than the control (196). This explanation is supported by subsequent results obtained using intracellular microelectrodes. None of the drugs studied, either alone or in combination, ever increases the amplitude of the intracellularly recorded action potentials in individual muscle fibres.

VII. SUMMARY AND CONCLUSIONS

1. The effects of some central depressants (morphine, methadone, propoxyphene, meperidine, naltrexone, naloxone, dextromethorphan, tetrodotoxin and procaine) on certain features of the active membrane related to sodium conductance in skeletal muscle were examined.
2. Studies with extracellular electrodes showed that increasing concentrations of these drugs caused a progressive depression of both the amplitude of the compound action potential and the excitability of the muscle fibres.
3. With all the drugs studied, the depressed responses were reversed to the control level when the preparation was brought back to the normal Ringer's fluid.
4. Intracellular recordings showed that the resting membrane potential remained essentially unchanged in the drug treated muscles.
5. The maximum rate of rise of the action potential was decreased by all the drugs. For propoxyphene, methadone, morphine and meperidine this effect was antagonized by increasing the extracellular sodium concentration or by having present in the bathing medium, low concentrations of opiate antagonists (e.g. naloxone or naltrexone). High concentrations of naloxone and naltrexone also depressed the maximum rate of rise. This effect could be antagonized for naloxone only by having present in the bathing medium, low concentrations of the other opiate antagonist (naltrexone). The depressant effect of procaine, tetrodotoxin and dextromethorphan on these parameters could not be antagonized by low concentrations of opiate antagonists but could be antagonized by increasing the extracellular sodium concentration.
6. The results of this study suggest that the central depressant

agents studied, block electrical excitability of the sartorius muscle of the frog, by suppressing the specific increase of sodium conductance, which normally follows depolarization of the fibre membrane.

7. Naloxone and naltrexone behaved as antagonists at low concentrations (10^{-8} - 10^{-7} M) but as agonists at high concentrations (10^{-4} - 10^{-3} M). The latter contradicts reports in the literature (108) which suggest that these opiates are 'pure' antagonists. Our results indicate that both naloxone and naltrexone are probably partial agonists with a very low intrinsic activity.

8. Opiate agonists (e.g. morphine, methadone and propoxyphene) produced a biphasic depression in the maximum rate of rise, similar to that of meperidine (56). Only the second component of this biphasic depression, which is probably due to a specific depression in \bar{g}_{Na} could be antagonized by low concentrations of opiate antagonists. The first component which also is elicited by procaine, could not be antagonized by opiate antagonists.

9. It is suggested that although the depressant effects of opiates and procaine appear to be identical, they are in fact mediated by two different mechanisms. Opiates mediate some of their depressant effects via interaction at specific opiate "receptors", whereas procaine, tetrodotoxin and dextromethorphan probably mediate their effects through other means.

10. The physiological role of the opiate receptors on frog sartorius muscle membrane, remains elusive.

11. Results of this investigation suggest that the 'receptor' sites for the opiates under investigation are located on the inner surface of the plasma membrane.

12. An overall impression gained from the results of this investigation is that there are opiate drug 'receptors' located on the inner surface of the muscle membrane associated with the 'sodium channels' and that drug activation of these receptors interferes with the opening of the "sodium channels" normally produced by membrane depolarization.

FUTURE CONSIDERATIONS

The results of this study suggest several avenues for future research.

Since the conclusion of this thesis is that the opiate receptors on frog sartorius muscle are intracellular, quaternary opiates could be employed to test this contention. Such studies may be supplemented with microiontophoretic application of opiates to either side of the plasma membrane. Another possibility that could be explored is the use of immunofluorescence. Immunohistochemical studies have been employed in the CNS to determine the cellular location of opiate receptors.

Lanthanum (La^{+++}) has been shown to substitute for, and calcium (Ca^{++}) to antagonize the effects of opiates in the CNS. It would thus be interesting to see whether these effects also occur in the sartorius muscle. If so, then other important studies, like the interaction between naloxone and La^{+++} , Ca^{++} and La^{+++} and Ca^{++} and naloxone will have to be conducted.

Endorphins have been shown in the CNS to substitute for and mimic the effects of opiates. These drugs should be studied in the sartorius muscle particularly their interaction effects with opiate agonists, antagonists, Ca^{++} and La^{+++} .

Another approach that merits investigation is to study the effects of pairs of optical isomers of both opiate agonists and antagonists. This should shed more light on the stereospecificity of the opiate receptor on frog sartorius muscle.

Since calcium is known to be intimately involved in some of the effects of opiates another interaction worth studying would be that

between opiates, calcium, calcium antagonists and calcium ionophores.

BIBLIOGRAPHY

1. Bernstein, J. (1902). Untersuchungen zur thermodynamik der bio-
cicktrischen Strome. Pflugers Arch. ges. Physiology.
92: 521.
2. MaCallum, A.B. (1905). On the distribution of potassium in an-
imal and vegetable cells. J. Physiol. 32: 95.
3. Boyle, P.J. & Conway, E.J. (1941). Potassium accumulation in
muscle and associated changes. J. Physiol. 100: 1.
4. Ussing, H.H. (1949). Transport of ions across cellular mem-
branes. Physiol. Rev. 29: 127.
5. Caldwell, P.C. (1960). The phosphorous metabolism of squid
axons and its relationship to the active transport of
sodium. J. Physiol. 152: 545.
6. Cole, K.S. & Curtis, H.J. (1939). Electric impedance of the
squid giant axon during activity. J. Gen. Physiol.
22p: 649.
7. Hodgkin, A.L. & Horowicz, P. (1959). Movements of Na^+ and K^+
in single muscle fibres. J. Physiol. 145: 405.
8. Nastuk, W.L. & Hodgkin, A.L. (1950). The electrical activity
of the single muscle fibres. J. Cell. comp. Physiol.
35: 39.
9. Hodgkin, A.L., Huxley, A.F. & Katz, B. (1949). Ionic currents
underlying activity in the giant axon of the squid.
Arch. Sci. Physiol. 3: 129.
10. Hodgkin, A.L. (1951). The ionic basis of electrical activity
in nerve & muscle. Biol. Rev. 26: 339.
11. Hodgkin, A.L. & Huxley, A.F. (1952). A quantitative description
of membrane current and its application to conduction
and excitation in nerve. J. Physiol. 117: 500.
12. Hodgkin, A.L. (1964). The conduction of the nervous impulse.
Liverpool: University Press.
13. Hodgkin, A.L. (1958). The Croonian Lecture: Ionic movements
and electrical activity in giant nerve fibres. Proc.
R. Soc. B. 148: 1.
14. Hille, B. (1970). Ionic channels in nerve membranes. Progr.
Biophys. 21: 1.
15. Desmedt, J.E. (1953). Electrical activity and intracellular
Sodium concentration in frog muscle. J. Physiol.
121: 191.

16. Lorente, de No, R. (1949). On the effect of certain quaternary ammonium ions upon frog nerve. *J. Cell. Comp. Physiol.* 33: (Suppl.) 1.
17. Fatt, P. & Katz, B. (1953). The electrical properties of crustacean muscle fibres. *J. Physiol.* 120: 171.
18. Bulbring, E. & Kuriyama, H. (1963). Effects of changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of guinea pig taenia coli. *J. Physiol.* 166: 29.
19. Schwartz, A., Lindenmayer, G.E. & Allen, J.C. (1975). The Sodium Potassium Adenosine Triphosphatase-Pharmacological, physiological and biochemical aspects. *Pharmacol. Rev.* 27: 1 March.
20. Smythies, J.R., Benington, F., Bradley, R.J., Bridgers, W.F. & Morin, R.D. (1974). The molecular structure of the sodium channel. *J. Theor. Biol.* 43: 29-42.
21. Armstrong, C.M. & Benzanilla, F. (1973 b). Currents related to the movement of gating particles of the sodium channels. *Nature, Lond.* 242: 459-461.
22. Hodgkin, A.L. & Keynes, R.D. (1955). The potassium permeability of a giant nerve fibre. *J. Physiol., Lond.* 128: 61-88.
23. Keynes, R.D. (1951). The ionic movements during nervous activity. *J. Physiol. (London)* 114: 119-150.
24. Baker, P.F., Hodgkin, A.L. & Ridgway, E.B. (1971). Depolarization and calcium entry in squid giant axons. *J. Physiol. (London)* 218: 709-755.
25. Narahashi, T., Deguchi, T., Urakawa, N. & Ohkubo, T. (1960). Stabilization and rectification of muscle fibre membrane by tetrodotoxin. *Am. J. Physiol.* 198: 934-938.
26. Lillie, R.S. (1923). Protoplasmic action and nervous action. pp. 206-216. Chicago, Illinois: The University of Chicago Press.
27. Thesleff, S. (1956). The effect of anesthetic agents on skeletal muscle membrane. *Acta Physiol. Scand.* 37: 335.
28. Shanes, A.M. (1958). Electrochemical aspects of physiological and pharmacological actions in excitable cells. *Pharmac. Rev.* 10: 165.

29. Blaustein, M.P. & Goldman, D.E. (1966). Action of anionic and cationic nerve-blocking agents: experiment and interpretation. *Science* 153: 429-432.
30. Narahashi, T., Frazier, D.T. & Yamada, M. (1970). The site of action and active form of local anesthetics. I. Theory and pH experiments with tertiary compounds. *J. Pharmacol. Exp. Ther.* 171: 32-44.
31. Narahashi, T., Frazier, D.T., Deguchi, T., Cleaves, C.A. & Ernau, M.C. (1971). The active form of pentobarbital in squid giant axons. *J. Pharmacol. Exp. Ther.* 177: 25-33.
32. Larrabee, M.G. & Posternak, J.M. (1952). Selective action of anesthetics on synapses and axons in mammalian sympathetic ganglia. *J. Neurophysiol.* 15: 91-114.
33. Mueller, P. & Rudin, D.O. (1967). Action potential phenomena in experimental bimolecular lipid membranes. *Nature (London)* 213: 603-604.
34. Allison, A.C. & Nunn, J.F. (1968). Effects of general anesthetics on microtubules. A possible mechanism of anesthesia. *Lancet*, 1326-1329.
35. Allison, A.C., Hulands, G.H., Nunn, J.F., Kitching, J.A., & MacDonald, A.C. (1970). The effect of inhalational anesthetics on the microtubular system in actinosphaerium nucleofilum. *J. Cell. Sci.* 1: 483-499.
36. Nunn, J.F., Lovis, J.D. & Kimball, K.L. (1971). Arrest of mitosis by halothane. *Brit. J. Anesth.* 43: 524-530.
37. Quastel, J.H. (1956). Action of drugs on enzyme systems. In *Enzymes: Units of Biological Structure & Function*, ed. by O.H. Gaebler. pp. 523-535. Academic Press, New York.
38. Larrabee, M.G. & Bronk, D.W. (1952). Metabolic requirements of sympathetic neurons. *Cold Springs Harbor Symp. Quant. Biol.* 17: 245-266.
39. Larrabee, M.G. & Holaday, D.A. (1952). Depression of transmission through sympathetic ganglia during general anesthesia. *J. Pharmacol. Exp. Ther.* 105: 400-408.
40. Larrabee, M.G., Ramos, J.G. & Bulbring, E. (1952). Effects of anesthetics on oxygen consumption and on synaptic transmission in sympathetic ganglia. *J. Cell. Comp. Physiol.* 40: 461-493.

41. Straub, R. (1956 a). Effects of local anesthetics on resting potential of myelinated nerve fibres. *Experientia* 12: 182.
42. Taylor, R.E. (1959). Effect of procaine on electrical properties of squid axon membrane. *Amer. J. Physiol.* 196: 1071.
43. Shanes, A.M., Freygang, W.H., Grundfest, H. & Amatneik, E. (1959). Anesthetic and calcium action in the voltage clamped squid giant axon. *J. Gen. Physiol.* 42: 793.
44. Inoue, F. & Frank, G.B. (1962). Action of procaine on frog skeletal muscle. *J. Pharmac. Exp. Ther.* 136: 190.
45. Inoue, F. & Frank, G.B. (1965). Action of ether on frog skeletal muscle. *Can. J. Physiol. Pharmacol.* 43: 751.
46. Inoue, F. & Frank, G.B. (1967). Effects of ethyl alcohol on excitability and on neuromuscular transmission in frog skeletal muscle. *Br. J. Pharmac. Chemother.* 30: 186.
47. Frank, G.B. & Sanders, H.D. (1963). A proposed common mechanism of action for general and local anesthetics in the central nervous system. *Br. J. Pharmac. Chemother.* 21: 1.
48. Uehara, Y. (1958). Conduction of nervous impulses in NaCl - deficient media. *Jap. J. Physiol.* 8: 282-291.
49. Uehara, Y. (1959). Graded nodal response in NaCl - deficient media, and the critical NaCl-concentration for "excitation". *Jap. J. Physiol.* 9: 304-310.
50. Uehara, Y. (1960). Narcotic and NaCl-deficiency as blocking agents. *Jap. J. Physiol.* 10: 267-274.
51. Yamaguchi, T. (1961). Electrophysiological studies on the mechanism of effects of anesthetics on the isolated frog muscle fibre. *J. Fac. Sci., Hokkaido Univ., Ser. VI, Zool.*, 14: 522.
52. Yamaguchi, T. & Okumura, H. (1963). Effects of anesthetics on the electrical properties of the cell membrane of the frog muscle fibre. *Arnot. Zool. Jap.* 36: 109.
53. Frank, G.B. & Pinsky, C. (1966). Tetrodotoxin-induced central nervous system depression. *Br. J. Pharmac. Chemother.* 26: 435.

54. Inoue, F., Pinsky, C. & Sanders, H.D. (1967). A comparison of the effects of hexa fluordiethyl ether and pentylenetetrazol on the excitability of frog skeletal muscle fibres. *Can. J. Physiol. Pharmacol.* 45: 757.
55. More, J.W., Blaustein, M.P., Anderson, N.C. & Narahashi, T. (1967). Basis of tetrodotoxin selectivity in blockage of squid axons. *J. Gen. Physiol.* 50p: 1401.
56. Frank, G.B. (1975). Two mechanisms for the meperidine block of action potential production in frog's skeletal muscle; non-specific and opiate drug receptor mediated blockade. *J. Physiol.* 252: 585-601.
57. Armstrong, C.M. & Binstock, L. (1964). The effects of several alcohols on the properties of the squid giant axon. *J. Gen. Physiol.* 48: 265-277.
58. Hille, B. (1966). Common mode of action of three agents that decrease the transient change in sodium permeability in nerves. *Nature (London)* 210: 1220-1222.
59. Kishimoto, U. & Adelman, Jr., W.J. (1964). Effect of detergent on electrical properties of squid axon membrane. *J. Gen. Physiol.* 47: 975-986.
60. Crescitelli, F. (1952). Modification in response to sodium of nerve fibres treated with drugs. *Am. J. Physiol.* 169: 638.
61. Condouris, G.A. (1961). A study of the mechanism of action of cocaine on amphibian peripheral nerve. *J. Pharmac. Exp. Ther.* 131: 243.
62. Shakalis, A. & Condouris, G.A. (1967). Local anesthetic action of cocaine on nerves from adrenalectomized frog. *Arch. Int. Pharmacodyn. Ther.* 166: 93.
63. Weidmann, S. (1955). Effects of calcium ions and local anesthetics on electrical properties of purkinje fibres. *J. Physiol.* 129: 568.
64. Lowenstein, E., Hallowell, P., Levine, F.H., Daggett, W.M., Austen, G. & Laner, M.B. (1969). Cardiovascular response to large doses of intravenous morphine in man. *New Eng. J. Med.* 281: 1389-1393.
65. Hasbrouck, M.D. (1971). The antagonism of morphine anesthesia by naloxone. *Anesth. Analg. Curr. Res.* 50: 954-959.

66. Snyder, S.H. (1975). The Opiate Receptor: The F.O. Schmitt Lecture in Neuroscience 1974 in Neurosciences Research Program Bulletin, 13: Supplement (August 1975).
67. Seeman, P. (1972). The membrane actions of anesthetics & tranquilizers. Pharmac. Rev. 24: 583-655.
68. Jacobsen, A.E. (1972). Narcotic analgesics and antagonists, Chemical and Biological Aspects of Drug Dependence (eds. Mule, S.J. & Brill, H.). The Chemical Rubber Co. Press, Cleveland, Ohio (1972), 101-118.
69. Portoghese, P.S. (1970). Relationships between stereo structure and pharmacological activity. Ann. Rev. Pharm. 10: 51-76.
70. Pohl, J. (1915). Uber das N-allylnorcodein einen antagonist des morphins, 2. Exp. Path. Ther. 17: 370-382.
71. Seevers, M.H. & Deneau, G.A. (1963). Physiological aspects of tolerance and physical dependence, 565-640. Physiological Pharmacology 1: (eds. Root, W.S. & Hoffman, F.G.), Academic Press, New York.
72. Villarreal, J.E. (1973). The effects of morphine agonists and antagonists on morphine-dependent Rhesus monkeys, In: Agonist and Antagonist Actions of Narcotic Analgesic Drugs (eds: Kosterlitz, H.W., Collier, H.O.J. & Villarreal, J.E.). University Park Press, 73-93.
73. Terenius, L. & Wahlstrom, A. (1975). Morphine like ligand for Opiate Receptors in Human C.S.F. Life Sciences 16: 1759-1764.
74. Snyder, S.H. (1975). Opiate receptor in normal and drug altered brain function. Nature 257: 185.
75. Goldstein, A., Goldstein, J.S. & Cox, B.M. (1975). A synthetic peptide with morphine-like pharmacologic action. Life Sciences 17: 1643-1654.
76. Hughes, J., Smith, T.W., Kosterlitz, H.W., Fothergill, L.A., Morgan, B.A. & Morris, H.R. (1975). Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258: 577.
77. Snyder, S.H., Pert, C.B. & Pasternak, G.W. (1974). The Opiate Receptor. Annals of Internal Medicine 81: 534-540.

78. Terenius, L. (1975). Comparison between Narcotic "Receptors" in the Guinea pig ileum and the Rat Brain. *Acta Pharmacol. et. toxicol.* 37: 211-221.
79. Terenius, L. & Wahlstrom, A. (1975). Search for an Endogenous ligant for the Opiate Receptor. *Acta Physiol. Scand.* 94: 74-81.
80. Goldstein, A. (1974). Opiate Receptors. *Life Sciences* 14: 615-623.
81. Goldstein, A., Aronow, L. & Kalman, S.M., eds (1974). Principles of Drug Action. The Basis of Pharmacology 2: New York, John Wiley & Sons.
82. Simon, E.J., Hiller, J.M. & Edelman, I. (1973). Stereospecific binding of the potent narcotic analgesic (³H) etorphine to rat-brain homogenate. *Proc. Nat. Acad. Sci.* 70: 1947-1949.
83. Simon, E.J., Hiller, J.M., Edelman, I., Groth, J. & Stahl, K.D. (1975). Opiate Receptors and their interactions with agonists and antagonists. *Life Sci.* 16: 1795-1800.
84. Paton, W.D.M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea pig ileum. *Br. J. Pharmacol. Chemother.* 12: 119-127.
85. Creese, I. & Snyder, S.H. (1975). Receptor binding and pharmacological activity in the guinea pig intestine. *J. Pharmacol. Exp. Ther.* 194: no. 1, 205-219.
86. Nickerson, M. (1956). Receptor occupance and tissue response. *Nature* 178: 697-698.
87. Ariens, E.J., van Rossum, J.M. & Simonis, A.M. (1957). Affinity, intrinsic activity and drug interactions. *Pharmac. Rev.* 9: 218-236.
88. Ariens, E.J., van Rossum, J.M. & Koopman, P.C. (1960). Receptor reserve and threshold phenomena. I. Theory and experiments with autonomic drugs tested on isolated organs. *Arch. Int. Pharmacodyn. Ther.* 127: 459-478.
89. Stephenson, R.P. (1956). A modification of receptor theory. *Brit. J. Pharmacol. Chemother.* 11: 379-478.
90. Martin, W.R. (1963). Analgesic & antipyretic drugs--strong analgesics. In: Physiological Pharmacology 1. The nervous system, Part A: Central Nervous System Drugs (eds. Root, W.S. and Hoffman, F.G.), Academic Press, New York. 275-312.

91. Smits, S. & Takemori, A.E. (1972). Studies on the quantitative antagonism of analgesia by naloxone and diprenorphine. *Eur. J. Pharmacol.* 20: 85-92.
92. Monod, J., Changeux, J.P. & Jacob, F. (1963). Allosteric proteins and cellular control systems. *J. Mol. Biol.* 6: 306-329.
93. Colquhoun, D. (1973). The relation between classical and co-operative models for drug action. In: Drug Receptors (ed. Rang, H.P.) University Park Press, 144-182.
94. Cox, B.M. & Weinstock, M. (1966). The effect of analgesic drugs on the release of acetylcholine from electrically stimulated guinea pig ileum. *Br. J. Pharm. Chemother.* 27: 81-92.
95. Cox, B.M. & Weinstock, M. (1966 b). Quantitative studies of the antagonism by nalorphine of some of the actions of morphine like analgesic drugs. *Br. J. Pharm. Chemother.* 27: 81-92.
96. Kosterlitz, H.W. & Watt, A.J. (1968). Kinetic parameters of narcotic agonists and antagonists with particular reference to N-allylnoroxymorphone (naloxone). *Br. J. Pharmacol.* 33: 266-276.
97. Takemori, A.E., Kupferberg, H.J. & Miller, J.W. (1969). Quantitative studies of the antagonism of morphine by nalorphine and naloxone. *J. Pharmacol. Exp. Ther.* 169: 39-45.
98. Pert, C.B., Pasternak, G. & Snyder, S.H. (1973). Opiate agonists and antagonists discriminated by receptor finding in brain. *Science* 182: 1359-1361.
99. Pert, C.B. & Snyder, S.H. (1974). Opiate receptor binding agonists and antagonists affected differentially by sodium. *Mol. Pharmacol.* 10: 868-879.
100. Snyder, S.H., Pasternak, G.W. & Pert, C.B. (1975). Opiate receptor mechanisms. In: Handbook of Psychopharmacology. Eds. Iversen, L.L., Iversen, S. and Snyder, S.H. New York: Plenum Press.
101. Pasternak, G.W. & Snyder, S.H. (1975). Opiate receptor binding: Enzymatic treatments that discriminate between agonist and antagonist interactions. *Mol. Pharmacol.* 11: 478-484.

102. Karlin, A. (1967). On the application of "a plausible model" of allosteric proteins to the receptor for acetylcholine. *J. Theor. Biol.* 16: 306-320.
103. Young, A.B. & Snyder, S.H. (1974 a). The glycine synaptic receptor: evidence that strychnine binding is associated with the ionic conductance mechanism. *Proc. Nat. Acad. Sci.* 71: 4002-4005.
104. Young, A.B. & Snyder, S.H. (1974 B). Strychnine binding in rat spinal cord membranes associated with the synaptic glycine receptor: cooperativity of glycine interactions. *Mol. Pharmacol.* 10: 790-809.
105. Pasternak, G.W. & Snyder, S.H. (1974). Opiate receptor binding; effects of enzymatic treatments. *Mol. Pharmacol.* 10: 183-193.
106. Wilson, H.A., Pasternak, G.W. & Snyder, S.H. (1975). Differentiation of opiate agonist and antagonist receptor binding by protein modifying reagents. *Nature* 253: 448-450.
107. Pasternak, G.W., Wilson, H.A. & Snyder, S.H. (1975). Differential effects of protein modifying reagents on receptor binding of opiate agonists and antagonists. *Mol. Pharmacol.* 11: 340-351.
108. Martin, W.R. (1967). Opioid antagonists. *Pharmacol. Rev.* 19: 463-521.
109. Pert, C.B., Aposhian, D. & Snyder, S.H. (1974 a). Phylogenetic distribution of opiate receptor binding. *Brain Res.* 75: 356-361.
110. Pert, C.B., Snowman, A.M. & Snyder, S.H. (1974 B). Localization of opiate receptor binding in synaptic membranes of rat brain. *Brain Res.* 70: 184-188.
111. Hughes, J. (1975). Search for the endogenous ligand of the opiate receptor. *Neurosciences Res. Prog. Bull.* 13: 55-58.
112. Pasternak, G.W. & Snyder, S.H. (1975). Identification of novel high affinity opiate receptor binding in rat brain. *Nature* 253: 563-565.
113. Terenius, L. (1975). Narcotic receptors in guinea pig ileum and rat brain. *Neurosci. Res. Prog. Bull.* 13: no. 1
In: Opiate Receptor Mechs Eds. S.H. Snyder & S. Matthysse.

114. Hughes, J. (1975). Search for the Endogenous ligand of the opiate receptor. *Neurosci. Res. Prog. Bull.* 13: no. 1 In: Opiate Receptor Mechs Eds. S.H. Snyder & S. Matthysse.
115. Mayer, D.J. (1975). Pain inhibition by electrical brain stimulation: comparison to morphine. *Neurosci. Res. Prog. Bull.* 13: no. 1. In: Opiate Receptor Mechs. Eds. S.H. Snyder & S. Matthysse.
116. Kosterlitz, H.W. (1975). Opiate actions in guinea pig ileum and mouse vas deferens. *Neurosci. Res. Prog. Bull.* 13: 68-73. In: Opiate Receptor Mechs. Eds. S.H. Snyder & S. Matthysse.
117. Jacob, J.J., Tremblay, E.C. & Colombel, M.C. (1974). Enhancement of nociceptive reactions by naloxone in mice and rats. *Psychopharmacologia* 37: 217-223.
118. Pert, C.B. & Snyder, S.H. (1973 a). Opiate receptors. Demonstration in nervous tissue. *Science* 179: 1011-1014.
119. Fahn, S. & Cote, L.J. (1968). Regional distribution of gamma-aminobutyric acid in brain of the rhesus monkey. *J. Neurochem.* 15: 209-213.
120. Hiller, J.M., Pearson, J. & Simon, E.J. (1973). Distribution of stereospecific binding of the potent narcotic analgesic etorphine in the human brain. Predominance in the limbic system. *Res. Commun. Chem. Pathol. Pharmacol.* 6: 1052-1062.
121. Kuhar, M.J., Pert, C.B. & Snyder, S.H. (1973). Regional distribution of the opiate receptor binding in monkey and human brain. *Nature* 245: 447-450.
122. Clonet, D. (ed.) (1971). *Narcotic drugs: Biochemical Pharmacology*, Plenum Press, New York.
123. Yamamura, H.I. & Snyder, S.H. (1974). Muscarinic cholinergic binding in rat brain. *Proc. Nat. Acad. Sci.* 71: 1725-1729.
124. Zukin, S.R., Young, A.B. & Snyder, S.H. (1974). Gamma-aminobutyric acid binding to receptor sites in the rat central nervous system. *Proc. Nat. Acad. Sci.* 71: 4802-4807.
125. Collier, H.O.J. & Roy, A.C. (1974). Morphine like drugs inhibit the stimulation by E prostaglandins of cyclic AMP formation by rat brain homogenate. *Nature* 248: 24-27.

126. Simon, E.J. (1975). Opiate receptor binding with ³H-Etorphine, In: Opiate Receptor Mechanisms, Neurosciences Research Program Bulletin 13: no. 1, February.
127. Lowney, L.I., Schulz, K., Lowery, P.J. & Goldstein, A. (1974). Partial purification of an opiate receptor from mouse brain. Science 183: 749-753.
128. Loh, H.H., Cho, T.M., Wu, Y.C. & Way, E.L. (1974). Stereo-specific binding of narcotics to brain cerebroside. Life Sci. 14: 2231-2245.
129. Loh, H.H. (1975). Cerebroside. In: Opiate Receptor Mechanisms, Neurosciences Research Program Bulletin 13: no. 1, February.
130. Iversen, L.L. (1975). Future Research. Discussion Review. In: Opiate Receptor Mechanisms, Neurosciences Research Program Bulletin 13: no. 1, February.
131. Nauta, W.J.H. (1975). Anatomical organization of pain pathways in the central nervous system. In: Opiate Receptor Mechanisms, Neurosciences Research Program Bulletin 13: no. 1, February.
132. Pert, A. (1975). Analgesia produced by morphine microinjections in the primate brain. In: Opiate Receptor Mechanisms, Neurosciences Research Program Bulletin 13: no. 1, February.
133. Goldstein, A. & Bloom, F.E. (1975). Biochemical phenomena in Opiate Action and Addiction. In: Opiate Receptor Mechanisms, Neurosciences Research Program Bulletin 13: no. 1, February.
134. Wikler, A. (1973). Theories related to physical dependence. In: Chemical and Biological Aspects of Drug Dependence (ed. Mule, S.J. & Brill, M.D.) The Chemical Rubber Co., 359-378.
135. Collier, H.O.J. (1965). A general Theory of the genesis of drug dependence by induction of receptors. Nature 205: 181-182.
136. Cochin, J. (1970). Possible mechanisms in development of tolerance. Fed. Proc. 29: 19-27.
137. Ehrenpreis, S. & Teller, D.N. (1973). Interaction of drugs of dependence with receptors. In: Chemical and Biological Aspects of Drug Dependence (eds. Mule, S.J. & Brill, M.D.) The Chemical Rubber Co. 177-218.

138. Pert, C.B. & Snyder, S.H. (1974). Opiate receptor binding of agonists and antagonists affected differentially by sodium. *Mol. Pharmacol.* 10: 868-879.
139. Snyder, S.H., Pasternak, G.W. & Pert, C.B. (1975). Opiate receptor mechanisms. In: *Handbook of Psychopharmacology*. Iversen, L.L., Iversen, S., & Snyder, S.H., eds. New York, Plenum Press.
140. Hitzemann, R.J. & Loh, H.H. (1975). On the use of tryptic digestion to localize narcotic binding material. *Life Scies.* 16: 1809-1810.
141. Craviso, G.L. & Musacchio, J.M (1975). Competitive inhibition of stereospecific opiate binding by local anesthetics in mouse brain. *Life Scies.* 16: 1803-1808.
142. Frazier, D.T., Narahashi, T. & Yamada, M. (1970). The site of action and active form of local anesthetics. II. Experiments with quaternary compounds. *J. Pharmacol. Exptl. Therap.* 171: 45-51.
143. Hughes, J. (1975). Isolation of an endogenous compound from the brain with pharmacological properties similar to morphine. *Brain Res.* 88: 295-308.
144. Terenius, L. & Wahlstrom (1974). Inhibitor(s) of narcotic receptor binding in brain extracts and cerebrospinal fluid. *Acta Pharmacol. Toxicol.* 35: (Suppl.) 55 (Abstr.).
145. Beckett, A.H. & Casy, A.F. (1954 a). Stereochemistry of certain analgesics. *Nature* 173: 1231-1232.
146. Beckett, A.H. & Casy, A.F. (1954 b). Synthetic analgesics stereochemical considerations. *J. Pharm. Pharmacol.* 6: 986-1001.
147. Lewis, J.W., Bentley, K.W. & Cowan, A. (1971). Narcotic analgesics and antagonists. *Ann. Rev. Pharmacol.* 11: 241-270.
148. Lasagna, L. (1954). Nalorphine (N-allylnormorphine). Practical and theoretical considerations. *Arch. Intern. Med.* 94: 532-558.
149. Burdette, B.H., Leeman, S. & Munson, P.L. (1961). The reversal by nalorphine of the inhibitory effect of morphine on the secretion of adrenocorticotrophic hormone in stress. *J. Pharmacol. Exp. Ther.* 132: 323-328.

150. Grumbach, L. & Chernov, H.I. (1965). The analgesic effect of opiate - opiate antagonist combinations in the rat. *J. Pharmacol. Exp. Ther.* 149: 385-396.
151. Cox, B.M. & Weinstock, M. (1964). Quantitative studies of the antagonism by nalorphine of some of the actions of morphine like analgesic drugs. *Brit. J. Pharmacol.* 22: 289-300.
152. Wikler, A. (1958). Opiates and opiate antagonists. A review of their mechanism of action in relation to clinical problems. *Public Health Monograph 52*, U.S. Gov. Printing Office, Washington.
153. Woods, L.A. (1956). The pharmacology of nalorphine (N-allylnormorphine), *Pharm. Rev.* 8: 175-198.
154. Mule, S.J., Redman, C.M. & Flesher, J.W. (1967). Intracellular disposition of ³H-morphine in the brain and liner of tolerant and non-tolerant guinea pigs. *J. Pharm. Exp. Ther.* 157: 459-471.
155. Martin, W.R. (1967). Opioid antagonists. *Pharmacol. Rev.* 19: 463-521.
156. Ariens, E.J., Simonis, A.M. & Van Rossum, J.M. (1964). Drug-receptor interaction. Interaction of one or more drugs with one receptor system. In: *Molecular Pharmacology. The Mode of Action of Biologically Active Compounds*. Ed. E.J. Ariens. Academic Press, New York, 119-286.
157. Smits, S.E. & Takemori, A.E. (1970). Quantitative studies on the antagonism by naloxone of some narcotic and narcotic-antagonist analgesics. *Brit. J. Pharmacol.* 39: 627-638.
158. Takemori, A.E., Hayashi, G. & Smits, S.E. (1972). Studies on the quantitative antagonism of analgesics by naloxone and diprenorphine. *Eur. J. Pharmacol.* 20: 85-92.
159. Schild, H.O. (1959). Drug antagonism and pAx. *Pharmacol. Rev.* 9: 242-246.
160. Portoghese, P.S. (1965). A new concept on the mode of interaction of narcotic analgesics with receptors. *J. Med. Chem.* 8: 609-616.
161. Takemori, A.E., Oka, T. & Nishiyama, N. (1973). Alteration of analgesic receptor antagonist interaction induced by morphine. *J. Pharmacol. Exp. Ther.* 186: 261-265.

162. Tulunay, F.C. & Takemori, A.E. (1974 a). The increased efficacy of narcotic antagonists induced by various narcotic analgesics. *J. Pharmacol. Exp. Ther.* 190: 395-400.
163. Tulunay, F.C. & Takemori, A.E. (1974 b). Further studies on the alteration of analgesic receptor-antagonist interactions induced by morphine. *J. Pharmacol. Exp. Ther.* 190: 401-407.
164. Takemori, A.E. (1974). Biochemistry of drug dependence. *Ann. Rev. Biochem.* 43: 15-33.
165. Goldstein, A., Lowney, L.I. & Pal, B.K. (1971). Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. *Proc. Nat. Acad. Sci.* 68: 1742-1747.
166. Pasternak, G.W. & Snyder, S.H. (1974). Opiate receptor binding. Effects of enzymatic treatments. *Mol. Pharmacol.* 10: 183-193.
167. Hollenberg & Cuatrecasas (1975, personal communication to S.H. Snyder) In *Opiate Receptor Mechanisms*, In; Handbook of Psychopharmacology, Iversen, L.L., Iversen, S. & Snyder, S.H., eds. New York: Plenum Press.
168. Pert, C.B. & Snyder, S.H. (1973). Properties of opiate receptor binding in rat brain. *Proc. Nat. Acad. Sci.* 70: 2243-2247.
169. Pert, C.B., Snowman, A.M. & Snyder, S.H. (1974). Localization of opiate receptor binding in synaptic membranes of rat brain. *Brain Res.* 70: 184-188.
170. Terenius, L. (1973 a). Characteristics of the "receptor" for narcotic analgesics in synaptic plasma membrane fraction from rat brain. *Acta Pharmacol. Toxicol.* 33: 317-320.
171. Terenius, L. (1973 b). Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. *Acta Pharmacol. Toxicol.* 32: 317-320.
172. Goldstein, A. (1974). Opiate receptors. *Life Sci.* 14: 615-623.
173. Pert, C.B. & Snyder, S.H. (1973 b). Properties of opiate-receptor binding in rat brain. *Proc. Nat. Acad. Sci.* 70: 2243-2247.
174. McClane, T.K. & Martin, W.R. (1967 a). Antagonism of the spinal cord effects of morphine and cyclazocine by naloxone and thebaine. *Int. J. Neuropharmacol.* 6: 325-327.

175. McClane, T.K. & Martin, W.R. (1967 b). Effects of morphine, nalorphine, cyclazocine and naloxone on the flexor reflex. *Int. J. Neuropharmacol.* 6: 89-98.
176. Curtis, D.R. & Duggan, A.W. (1969). On the existence of Renshaw Cells. *Brain Res.* 15: 597-599.
177. Korf, J., Bunney, B.S. & Aghajanian, G.K. (1974). Noradrenergic neurons: Morphine inhibition of spontaneous activity. *Eur. J. Pharmacol.* 25: 165-169.
178. Simon, E.J., Dole, W.P. & Hiller, J.M. (1972). Coupling of a new, active morphine derivative to sepharose for affinity chromatography. *Proc. Nat. Acad. Sci.* 69: 1835-1837.
179. Mayer, D.J., Wolfle, T.L., Akil, H., Carder, B. & Lieberkind, J.C. (1971). Analgesia from electrical stimulation in the brainstem of the rat. *Science* 174: 1351-1354.
180. Lieberkind, J.C., Guilbaud, G., Besson, J.M. & Oliveras, J.L. (1973). Analgesia from electrical stimulation of the periaqueductal gray matter in the cat: behavioral observations and inhibitory effects on spinal cord interneurons. *Brain Res.* 50: 441-446.
181. Mayer, D.J. & Lieberkind, J.C. (1974). Pain reduction by focal electrical stimulation of the brain. An anatomical and behavioral analysis. *Brain Res.* 68: 73-93.
182. Kosterlitz, H.W., Waterfield, A.A. & Berthoud, V. (1973). Assessment of the agonist and antagonist properties of narcotic analgesic drugs by their actions on the morphine receptor in the guinea pig ileum. In: *Advances in Biochemical Psychopharmacology 8: Narcotic Antagonists*. Braude, M.C., Harris, L.S., May, E.L., Smith, J.P. & Villarreal, J.E. eds. New York: Raven Press, 319-334.
183. Schaumann, W. (1957). Inhibition by morphine of the release of acetylcholine from the intestine of the guinea pig. *Brit. J. Pharmacol. Chemotherap.* 12: 115-116.
184. Trendelenburg, U. (1957). The action of morphine on the superior cervical ganglion and on the nictitating membrane of the cat. *Brit. J. Pharmacol. Chemotherap.* 12: 79-85.
185. Pelikan, E.W. (1960). The mechanism of ganglionic blockade produced by nicotine. *Ann. N.Y. Acad. Sci.* 90: 52-69.

186. Cairnie, A.B., Kosterlitz, H.W. & Taylor, D.W. (1961). Effect of morphine on some sympathetically innervated effectors. *Brit. J. Pharmacol. Chemotherap.* 17: 539-551.
187. Frederickson, R.C.A. & Pinsky, C. (1971). Morphine impairs acetylcholine release but facilitates acetylcholine action at a skeletal neuromuscular junction. *Nature N.B.* 231: 93-94.
188. Lees, G.M., Kosterlitz, H.W. & Waterfield, A.A. (1973). Characteristics of morphine-sensitive release of neurotransmitter substances. In: Agonist and Antagonist Actions of Narcotic Analgesic Drugs: Kosterlitz, H.W., Collier, H.O.J. and Villarreal, J.E., eds. University Park Press, Baltimore, Maryland. 142-152.
189. Frank, G.B. (1968). Drugs which modify membrane excitability. *Fed. Proc.* 27: 132-136.
190. Frank, G.B. (1972). The effects of anesthetic drugs based on alteration of membrane excitability. In: Cellular Biology and Toxicity of Anesthetics. B. Raymond Fink, ed. Williams & Wilins Co., Baltimore. 29-38.
191. Kosterlitz, H.W., & Wallis, D.I. (1964). The action of morphine-like drugs on impulse transmission in mammalian nerve fibres. *Br. J. Pharmacol.* 22: 499-510.
192. Seeman, P., Chau-Wong, M. & Moyyen, S. (1972). Absorption and conduction block of opiate narcotics on nerve membranes; identical effects of levo- and dextro-forms. *Can. J. Physiol. Pharmacol.* 50: 1181-1192.
193. Simon, E.J. & Rosenberg, P. (1970). Effects of narcotics on the giant axon of the squid. *J. Neurochem.* 17: 881-887.
194. Frazier, D.T., Murayama, N., Abbott, N.J. & Narahashi, T. (1972). Effects of morphine on internally perfused squid giant axons. *Proc. Soc. Exp. Biol. Med.* 139: 434-438.
195. Frank, G.B. & Buttar, H.S. (1975). Effects of morphine and meperidine on action potential production in frog's skeletal muscle fibres. *Can. J. Physiol. Pharmac.* 53: 92-96.
196. Frank, G.B. (1975). Opiate drug receptors on excitable cell membranes. *Arch. Int. Pharmacodyn.* 216.
197. Lowenstein, E., Hallowell, P., Levine, F.H., Daggett, W.M., Austen, G. & Laver, M.B. (1969). Cardiovascular response to large doses of intravenous morphine in

man. New Eng. J. Med 281: 1389-1393.

198. Karkalas, J. & Lal, H. (1973). A comparison of haloperidol with methadone in blocking heroin withdrawal symptoms. *Int. Pharmacopsychiat.* 8: 248-251.
199. Longo, V.G. (1972). Neuropharmacology and Behavior. W.H. Freeman & Co., San Francisco.
200. Dingledine, R. & Goldstein, A. (1975). Single Neuron studies of opiate action in the guinea pig myenteric plexus. *Life Sci.* 17: 57-62.
201. Satoh, M., Zieglgansberger, W. & Herz, A. (1975). Interaction between morphine and putative excitatory neurotransmitters in cortical neurons in naive and tolerant rats. *Life Sci.* 17: 75-80.
202. Kakunaga, T., Kaneto, H. & Hano, K. (1966). Pharmacological studies on analgesics. VII. Significance of the calcium ion in morphine analgesia. *J. Pharmacol. Exp. Ther.* 153: 134-141.
203. Kaneto, H. (1971). Inorganic ions: The role of calcium. In: Narcotic Drugs: Biochemical Pharmacology, ed. by D.H. Clouet. 300-309. Plenum Publishing Co., New York.
204. Rubin, R.P. (1970). The role of calcium in the release of neurotransmitter substances and hormones. *Pharmacol. Rev.* 22: 389-428.
205. Rubin, R.P. (1974). Calcium and the Secretory Process. Plenum Publishing Corporation, New York.
206. Shans, A.M. (1958). Electrochemical aspects of excitable cells. *Pharmacol. Rev.* 10: 59-273.
207. Manery, J.F. (1966). Effects of cations on membranes. *Fed. Proc.* 25: 1804.
208. Frankenhaeuser, B. and Hodgkin, A.L. (1957). The action of calcium on the electrical properties of squid axons. *J. Physiol. (London)* 137: 218-244.
209. Koketsu, K. & Nishi, S. (1969). Calcium and action potentials of bullfrog sympathetic ganglion cells. *J. Gen. Physiol.* 53: 608-620.
210. Rojas, E., Taylor, R.E., Atwater, I. & Bezanilla, F. (1969). Analysis of the effects of calcium or magnesium on voltage-clamp currents in perfused squid axons bathed in solns. of high potassium. *J. Gen. Physiol.* 54: 532-544.

211. Hurwitz, L. & Suria, A. (1971). The link between agonist action and response in smooth muscle. *Ann. Rev. Pharmacol.* 11: 303-326.
212. Chang, K.J. & Triggle, D.J. (1973). Quantitative aspects of drug receptor interactions. I. Ca^{2+} and cholinergic receptor activation in smooth muscle. A basic model for drug-receptor interactions. *J. Ther. Biol.* 40: 125-154.
213. Hyden, H. (1974). A calcium-dependent mechanism for synapse and nerve cell membrane modulation. *Proc. Nat. Acad. Sci. U.S.A.* 71: 2965-2968.
214. Landowne, D. & Ritchie, J.M. (1971). On the control of glycogenolysis in mammalian nervous tissue by calcium. *J. Physiol. (London)* 212: 503-517.
215. Rasmussen, H. & Nagata, N. (1970). Hormones, cell calcium and cyclic A.M.P. In: *Calcium & Cellular Function*. ed. by A.W. Cuthbert. 198-209. MacMillan, London.
216. Hitzemann, R.J., Hitzemann, B.A. & Loh, H.H. (1974). Binding of ^3H -naloxone in the mouse. Effect of ions and tolerance development. *Life Sci. Part I., Physiol. Pharmacol.* 14: 2393-2404.
217. Harris, R.A., Loh, H.H. & Way, E.L. (1975). Effects of divalent cations, cation chelators, and an inophore on morphine analgesia and tolerance. *J. Pharmacol. Exp. Ther.* 195: 488-498.
218. Kao, C.Y. & Nishiyama, A. (1964). Ovarian hormones and resting potential of rabbit uterine smooth muscle. *Am. J. Physiol.* 207: 793-799.
219. Hodgkin, A.L. & Katz, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* 108: 37-77.
220. Seeman, P. (1975). Action and interaction of psychoactive drugs on nerve impulse conduction and release of neurotransmitters, 165-170. In: *Clinical Pharmacology of Psychoactive Drugs*. Ed. by E.M. Sellers. Addiction Research Foundation of Ontario.
221. Satoh, M., Zieglgansberger, W., Fries, W. & Herz, A. (1974). Opiate agonist-antagonist interaction at cortical neurones of naive and tolerant-dependent rats. *Brain Res.* 82: 378-382.

222. Steick, T.L. (1972). The organization of proteins in human erythrocyte membranes, 71. In: Membrane Research. Academic Press, New York.
223. Tasaki, I., Polley, E.H. & Orrego, F. (1954). Action potential from individual elements in cat geniculate and striate cortex. J. Neurophysiol. 17: 454.
224. Narahashi, T., Anderson, N.C. & Moore, J.W. (1966). Tetrodotoxin does not block excitation from inside the nerve membrane. Science 153: 765-767.
225. Dole, V.P., Cuatrecasas, P., Goldstein, A. (1975). Biochemical identification of receptors. In: Opiate Receptor Mechanisms, Neurosciences Research Program Bulletin, V. 13, no. 1, February. Eds: Snyder, S.H. & Matthysse, S.
226. Cote, L., & Fahn, S. (1967). Some aspects of the biochemistry of the substantia nigra of the rhesus monkey. In: Progress in Neurogenetics, Vol. 1, pp. 311-317, Excerpta Medica International Congress Series, Amsterdam.
227. Frank, G.B. (1957). The effects of trematode infection on the physiological activity of the frog. Can. J. Biochem. Physiol. 35: 347.
228. Ritchie, J.M. (1975). Mechanism of action of local anesthetic agents and biotoxins. Br. J. Anaesth. (1975), 47, 191.
229. Metcalfe, J.C. (1971). Interaction of local anesthetics and calcium with erythrocyte membranes. In: A symposium on Calcium and Cellular Function, ed. by A.W. Cuthbert, pp. 219-240., MacMillan Co., London.
230. De Jong, R.H. & Wagman, I.H. (1963). Physiological mechanisms of peripheral nerve block by local anesthetics. Anesthesiology 24: 684-727.
231. Coombs, J.S., Eccles, J.C. & Fatt, P. (1955). The electrical properties of the motoneurone membrane. J. Physiol. 130: 291.
232. Eccles, J.C. (1953). The neurophysiological basis of mind. 1st ed., p. 4, London; Oxford Univ. Press.
233. Kosterlitz, H.W. & Hughes, J. (1975). Possible mechanisms for the action of enkephelin. Life Sciences 17: July 1, 1975, p. 94.

234. Strichartz, G. (1976). Molecular mechanisms of nerve block by local anesthetics. *Anesth.* 45: 421-441.
235. Hille, B. (1977). Ionic channels of nerve: questions for theoretical chemists. *BioSystems* 8: 195-199.
236. Narahashi, T. (1975). Neurotoxins: pharmacological dissection of ionic channels of nerve membranes. In: The Nervous System Vol. 1: The Basic Neurosciences, Raven Press, New York.
237. Rosen, M.R. (1977). Cellular electrophysiologic basis of cardiac arrhythmias. *Angiology* 28: 289-299.
238. Blaustein, M.P. (1968). Barbiturates block sodium and potassium conductance increases in voltage-clamped lobster axons. *J. Gen. Physiol.* 51: 293-307.
239. Greuner, R. & Narahashi, T. (1972). The mechanism of excitability blockade by chlorpromazine. *J. Pharmacol. Exp. Ther.* 181: 161-170.
240. Neuman, R.S., Inoue, F. & Frank, G.B. (1973). Effects of central nervous system depressants on voltage clamped myelinated nerve. *Can. Fed. Biol. Soc. (Abstr.)* 16: 379.
241. Moore, J.W., Ulbright, W. & Takata, M. (1964). Effect of ethanol on the sodium and potassium conductances of squid axon membrane. *J. Gen. Physiol.* 48: 279-295.
242. Pincus, J.H. (1972). Diphenylhydantoin and ion flux in lobster nerve. *Arch. Neurol.* 26: 4-10.
243. Wu, C.H. & Narahashi, T. (1973). Mechanism of action of propranolol on squid axon membranes. *J. Pharmacol. Exp. Ther.* 184: 155-162.
244. Lipicky, R.J., Gilbert, D.L. & Stillman, I.M. (1972). Diphenylhydantoin inhibition of sodium conductance in squid giant axon. *Proc. Natl. Acad. Sci.* 69: 1758-1760.
245. Frank, G.B. & Jhamandas, K. (1970). Effects of general depressant drugs on the electrical responses of isolated slabs of cat's cerebral cortex. *Br. J. Pharmacol.* 39: 707-715.
246. Ariens, E.J. & Simonis, A.M. (1976). Receptors and receptor mechanisms. In: Beta-Adrenoceptor Blocking Agents, eds., Saxena, P.R. & Forsyth, R.P. pp. 1-27. North-Holland Publishing Company, Amsterdam.
247. Ariens, E.J. & Beld, A.J. (1977). The receptor concept in evolution. *Biochem. Pharmacol.* 26: 913-918.

248. Peters, T. (1977). Membrane structure and drug actions. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 297: S1-S4.
249. Somlyo, A.V. & Somlyo, A.P. (1968). Vascular smooth muscle I. Normal structure, pathology, biochemistry, and biophysics. *Pharma. Rev.* 20: 197-272.
250. Triggle, D.J. (1971). Neurotransmitter-receptor Interactions. Academic Press, New York.
251. Triggle, D.J. (1972). Effects of calcium on excitable membranes and neurotransmitter action. *Prog. Surf. Mem. Sci.* 5: 267-331.
252. Knapp, S., Mandell, A.J. & Bullard, W.P. (1975). Calcium activation of brain tryptophan hydroxylase. *Life Sci.* 16: 1583-1594.
253. Ross, D.H., Lynn, S.C. & Cardenas, H.L. (1977). Ions, opiates and cellular adaptation. In: Alcohol and Opiates, pp. 265-281, Academic Press, New York.
254. Sanghvi, I.S. & Gershon, S. (1977). Brain calcium and morphine action. *Biochem. Pharmacol.* 26: 1183-1185.
255. Weiss, G.B. (1974). Cellular pharmacology of lanthanum. *Ann. Rev. Pharm.* 14: 343-354.
256. Harris, R.A., Loh, H.H. & Way, E.L. (1976). Antinociceptive effects of lanthanum and cerium in non-tolerant and morphine tolerant-dependent animals. *J. Pharm. exp. Ther.* 196: 288.
257. Harris, R.A., Iwamoto, E.T., Loh, H.H. & Way, E.L. (1975). Analgesic effects of lanthanum cross tolerance with morphine. *Brain Res.* 100: 221.
258. Kosterlitz, H.W., Lees, G.M., Wallis, D.I. & Watt, A.J. (1968). Non-specific inhibitory effects of morphine-like drugs on transmission in the superior cervical ganglion and guinea-pig isolated ileum. *Br. J. Pharmac.* 34: 691P.
259. Kosterlitz, H.W., Lees, G.M. & Watt, A.J. (1969). Non-specific effects of morphine-like drugs. *Pharmac. Res. Commun.* 1: 42-48.
260. Roth, S.H., Smith, R.A. & Paton, W.D.M. (1976). Pressure antagonism of anaesthetic-induced conduction failure in frog peripheral nerve. *Br. J. Anaesth.* 48: 621-628.
261. Kendig, J.J., Trudell, J.R. & Cohen, E.N. (1975). Effects of pressure and anesthetics on conduction and synaptic transmission. *J. Pharmacol. Exp. Ther.* 195: 216-224.

262. Kendig, J.J. & Cohen, E.N. (1977). Pressure antagonism to nerve conduction block by anesthetic agents. *Anesthesiology* 47: 6-10.
263. Johnson, F.H., Flagler, E.A. (1951). Activity of narcotized amphibian larvae under hydrostatic pressure. *J. Cell. Comp. Physiol.* 37: 15-25.
264. Halsey, M.J. & Wardley-Smith, B. (1975). Pressure reversal of narcosis produced by anaesthetics, narcotics and tranquilizers. *Nature* 257: 811-813.
265. Winter, P.M., Smith, R.A., Smith, M., et al. (1976). Pressure antagonism of barbiturate anesthesia. *Anesthesiology* 44: 416-419.
266. Richards, C.D. & Hesketh, T.R. (1975). Implications for theories of anaesthesia of antagonism between anaesthetic and non-anaesthetic steroids. *Nature* 256: 179-182.
267. Marwaha, J. & Frank, G.B. (1976). A study of the actions of two narcotic antagonists on the opiate drug receptor on frog's skeletal muscle fibre membranes. *Can. Fed. Biol. Soc. (Abstr.)* 19: 169.
268. Frank, G.B. & Marwaha, J. (1977). An investigation of the activity of opiate drug receptors located on the frog's skeletal muscle fibre membrane. (Submitted for publication).
269. Finck, A.D., Ngai, S.H. & Berkowitz, B.A. (1977). Antagonism of general anesthesia by naloxone in the rat. *Anesthesiology* 46: 241-245.
270. Berkowitz, B.A., Finck, A.D. & Ngai, S.H. (1976). Nitrous oxide analgesia and its reversal by narcotic antagonists. *Pharmacologist* 18: 177.
271. Berkowitz, B.A., Ngai, S.H. & Finck, A.D. (1976). Nitrous oxide "analgesia": resemblance to opiate action. *Science* 194: 967-968.
272. Goldstein, A. (1976). Opioid peptides (endorphins) in pituitary and brain. *Science* 193: 1081-1086.
273. Holmes, H.L. (1975). Structure Activity Relationships Vol. I. Defence Research Establishment Suffield, Ralston, Alberta.
274. Snyder, S.H. & Simantov, R. (1977). The opiate receptor and opioid peptides. *J. Neurochem.* 28: 13-20.

275. Birdsall, N.J.M., Bradbury, A.F., Burgen, A.S.V., Hulme, E.C., Smyth, D.G. & Snell, C.R. (1976). Interactions of peptides derived from the C-fragment of β -lipotropin with brain opiate receptors. *Proc. B.P.S.* 460P-461P.
276. Cardenas, H.L. & Ross, D.H. (1976). Calcium depletion of synaptosomes after morphine treatment. *Br. J. Pharm.* 57: 521-526.
277. Collier, H.O.J. (1973). *Proc. 5th Int. Congr. Pharmacol., San Francisco, Vol. 1.* Eds. Cochin, J. & Karger, B. pp. 65-76.
278. Waterfield, A.A. & Kosterlitz, H.W. (1975). Stereospecific increase by narcotic antagonists of evoked acetylcholine output in guinea pig ileum. *Life Sci.* 16: 1787-1792.
279. Jacob, J.J., Tremblay, E.C. & Colombel, M.C. (1974). Facilitation de reactions nociceptives par la naloxone chez la souris et chez le rat. *Psychopharmacologia* 37: 217-223.
280. Akil, H., Mayer, D.J. & Liebeskind, J.C. (1976). Antagonism of stimulated-produced analgesia by naloxone, a narcotic antagonist. *Science* 191: 961-962.
281. Jhamandas, K., Pinsky, C. & Phillis, J.W. (1970). Effects of morphine and its antagonists on release of cerebral cortical acetylcholine. *Nature* 228: 176-177.
282. Pasternak, G.W., Goodman, R. & Snyder, S.H. (1975). An endogenous morphine-like factor in mammalian brain. *Life Sci.* 16: 1765-1769.
283. Simantov, R., Kuher, M., Pasternak, G.W. & Snyder, S.H. (1976). The regional distribution of a morphine-like factor "enkephalin" in monkey brain. *Brain Res.* 111: 204-211.
284. Frederickson, R.C.A., Schirmer, E.W., Grinnan, E.L., Harrel, C. E. & Hewes, C.R. (1976). Human endorphin: comparison with porcine endorphin, enkephalin and normorphine. *Life Sci.* 19: 1181-1190.
285. Bradbury, A.F., Smyth, D.G. & Snell, C.R. (1975). Biosynthesis of β -MSH and ACTH. In: *Chemistry: Structure and Biology*, eds. Walter, R. & Meienhofer, J., pp. 609-615, Ann Arbor Sci. Inc.
286. Bradbury, A.F., Smyth, D.G. & Snell, C.R. (1976). Prohormones of β -melanotropin (β -melanocyte stimulating hormone, β -MSH) and corticotropin (adrenocorticotrophic hormone, ACTH): structure and activation. In: *Polypeptide Hormones: Molecular and Cellular Aspects*, pp. 61-75. Ciba

287. Bradbury, A.F., Feldberg, W.F., Smyth, D.G. & Snell, C.R. (1976). Lipotropin C-fragment. In: Opiates and Endogenous Opioid Peptides, ed Kosterlitz, H.W. pp 9-18, North Holland, Amsterdam.
288. Guillemin, R., Ling, N. & Burgus, R. (1976). Endorphines, peptides d'origine hypothalamique et neurohypophysaire a activite morphinomimetique. Isolement et structure moleculaire d' -endorphine, C.R. Hebd. Seanc. Acad. Sci. Paris D282, 783-785.
289. Simantov, R. & Snyder, S. (1976). Structure identification of a morphine-like peptide 'enkephalin' in bovine brain. Life Sci. 18: 781-788.
290. Schulz, R., Wuster, M. & Herz, A. (1977). Detection of a long acting endogenous opioid in blood and small intestine. Life Sci. 21: 105-116.
291. Waterfield, A.A. & Kosterlitz, H.W. (1975). Stereospecific increase by narcotic antagonists of evoked acetylcholine output in guinea-pig ileum. Life Sci. 16: 1787-1792.
292. Cox, B.M., Gentleman, S., Su, T. & Goldstein, A. (1976). Further characterization of morphine-like peptides (endorphins) from pituitary. Brain Res. 115: 285-286.
293. Goldstein, A. (1975). In: Biological and Behavioral Approaches to Drug Dependence, eds. Cappel, H. & LeBlanc, A.E. Proc. International Symposia on Alcohol and Drug Research, Addiction Research Foundation Toronto.
294. Elde, R., Hokfelt, T., Johansson, O. et al. (1976). Immunohistochemical studies using antibodies to leucine-enkephalin: initial observations on the nervous system of the rat. Neuroscience 1: 349-351.
295. Frederickson, R.C.A. (1977). Enkephalin pentapeptides - a review of current evidence for a physiological role in vertebrate neurotransmission. Life Sci. 21: 23-42.
296. Li, C.H. (1964). Lipotropin, a new active peptide from pituitary glands. Nature 201: 924.
297. Guillemin, R. (1977). Endorphins, brain peptides that act like opiates. New Eng. J. Med. 296: 226-228.
298. Goldstein, A. (1977). Future research on opioid peptides (endorphins): a review. In: Alcohol and Opiates pp. 397-403, Academic Press, New York.
299. Dey, P.K. & Feldberg, W. (1976). Analgesia produced by morphine when acting from the liquor space. Br. J. Pharmac. 58: 383-393.

300. Pert, C.B., Pert, A. & Tallman, J.F. (1976). Isolation of a novel endogenous opiate analgesic from human blood. *Proc. Natl. Acad. Sci. USA* 73: 2226-2230.
301. Ungar, G. & Ungar, A.L. (1976). Endogenous morphine antagonist in morphine-tested rat brain. *Fed. Proceeds* 35: 309.
302. Klee, W.A. (1977). Endogenous opiate peptides. In: *Peptides In Neurobiology*, ed. Gainer, H. pp. 375-396, Plenum Press.
303. Knoll, J. (1976). Neuronal peptide (enkephalin) receptors in the ear artery of the rabbit. *Eur. J. Pharmacol.* 39: 403-407.
304. Belluzzi, J.D. & Stein, L. (1977). Enkephalin may mediate euphoria and drive-reduction reward. *Nature* 266: 556-558.
305. Moroni, F., Cheney, D.L. & Costa, E. (1977). β endorphin inhibits ACh turnover in nuclei of rat brain. *Nature* 267: 267-268.
306. Baxter, M.G., Goff, D., Miller, A.A. & Saunders, I.A. (1977). Effect of a potent synthetic opioid pentapeptide in some antinociceptive and behavioural tests in mice and rats. *Proc. B.P.S.* pp. 455P-456P.
307. Dupont, A., Cusan, L., Garon, M., Labrie, F. & Li, C.H. (1977). β -endorphin: stimulation of growth hormone release in vivo. *Proc. Natl. Acad. Sci. USA* 74: 358-359.
308. Brandt, M., Gullis, R.J. *et al.* (1976). Enkephalin regulates the levels of cyclic nucleotides in neuroblastoma x glioma hybrid cells. *Nature* 262: 311-313, 1976.
309. Wei, E. & Loh, H.H. (1976). Physical dependence on opiate-like peptides. *Science* 193: 1262.
310. Loh, H.H., Tseng, L.F., Wei, E. & Li, C.H. (1976). β -endorphin is a potent analgesic agent. *Proc. Nat. Acad. Sci.* 73.
311. Smith, T.W., Hughes, J., Kosterlitz, H.W. & Sosa, R.P. (1976). Enkephalins: isolation, distribution and function. In: *Cellular Effects of Opiates*, ed Kosterlitz, H.W. pp. 57-62, North Holland Press, Amsterdam.
312. Kuhar, M.J., Pert, C.B. & Snyder, S.H. (1973). Regional distribution of opiate receptor binding in monkey and human brain. *Nature* 245: 447-451.
313. Hillér, J.M., Pearson, J. & Simon, E.J. (1973). Distribution of stereospecific binding of the potent narcotic analgesic etorphine in the human brain: predominance in the limbic system. *Res Commun. chem. path. Pharmacol.* 6: 1052-1061.

314. Simantov, R., Kuhar, M.J., Pasternak, G.W. & Snyder, S.H. (1976). The regional distribution of a morphine-like factor enkephalin in monkey brain. *Brain Res.* 106: 189-197.
315. Simantov, R., Snowman, A.M. & Snyder, S.H. (1976). A morphine-like factor "enkephalin" in rat brain: subcellular localization. *Brain Res.* 107: 650-657.
316. Queen, G., Pinsky, C. & LaBella, F.S. (1976). Subcellular localization of endorphine activity in pituitary and brain. *Biochem. Biophys. Res. Commun.* 72: 1021-1027.
317. Clouet, D. & Ratner, M. (1976). In: Opiates and Endogenous Opioid Peptides, ed. Kosterlitz, H.W., pp. 71-78, North Holland Press, Amsterdam.
318. Frederickson, R.C.A., Nickander, R., Smithwick, E.L., Shuman, R. & Norris, F.H. (1976). Pharmacological activity of Met-enkephalin and analogues in vitro and in vivo - depression of single neuronal activity on specified brain regions. In: Opiates and Endogenous Opioid Peptides, ed. Kosterlitz, H.W., pp. 239-246, North Holland Press, Amsterdam.
319. Hambrock, J.M., Morgan, B.A., Rance, M.J. & Smith, C.F.C. (1976). Mode of deactivation of enkephalins by rat and human plasma and rat brain homogenates. *Nature* 262: 782-783.
320. Frederickson, R.C.A., Smithwick, E. & Schuman, R. (unpublished) quoted in: Enkephalin pentapeptides - a review of current evidence for a physiological role in vertebrate neurotransmission. *Life Sci.* 21: 23-42.
321. Pert, C.B., Pert, A., Chang, J.K. & Fong, B.T.W. (1976). [D-Ala²]-met-enkephalinamide: a potent long lasting synthetic pentapeptide analgesic. *Science* 194: 331-332.
322. Yaksh, T.L., Frederickson, R.C.A., Huang, S.P. & Rudy, T.A. (unpublished). Quoted in: Enkephalin pentapeptides - a review of current evidence for a physiological role in vertebrate neurotransmission. *Life Sci.* 21: 23-42.
323. Pert, C.B., Bowie, D.L., Fong, B.T.W. & Chang, J.K. (1976). Synthetic analogues of Met-enkephalin which resist enzymatic destruction. In: Opiates and Endogenous Opioid Peptides, ed. Kosterlitz, H.W., pp. 79-86, North Holland Press, Amsterdam.
324. Puig, M.M., Gascon, P., Craviso, G.L., Bjur, R.A., Matsueda, G., Stewart, J.M. & Musacchio, J.M. (1977). The effect of enkephalin and enkephalin analogs on the guinea-pig ileum and rat brain opiate receptor. *Arch. int. Pharmacodyn.* 226: 69-80.

325. Van Nueten, J.M., Janssen, P.A.J. & Fontaine, J. (1976). Unexpected reversal effects of naloxone on the guinea pig ileum. *Life Sci.* 18: 803-810.
326. Schulz, R., Wuster, M., Simantov, R., Snyder, S. & Herz, A. (1977). Electrically stimulated release of opiate-like material from the myenteric plexus of the guinea pig ileum. *Eur. J. Pharmacol.* 41: 347-348.
327. Wahlstrom, A., Johansson, L. & Terenius, L. (1976). Characterization of endorphins (endogenous morphine-like factors) in human CSF and brain extracts. In: Opiates and Endogenous Opioid Peptides, ed. Kosterlitz, H.W. pp. 49-56, North Holland Press, Amsterdam.
328. Frederickson, R.C.A., Burgis, V. & Edwards, J.D. (1977). Diurnal rhythm in endorphin activity in mouse brain - effects of naloxone, pilocarpine and Bactofen. *Fed. Proc.* 36: 965.
329. Frederickson, R.C.A., Burgis, V., Edwards, J.D. & Harrell, C.E. (Unpublished). Quoted in: Enkephalin pentapeptides- a review of current evidence for a physiological role in vertebrate transmission. *Life Sci.* 21: 23-42.
330. Sjolund, B. & Eriksson, M. (1976). Electro-acupuncture and endogenous morphines. *The Lancet* Nov. 13, p. 1085.
331. Shaar, C.J., Frederickson, R.C.A., Dininger, N.B. (Unpublished). Quoted in: Enkephalin pentapeptides - a review of current evidence for a physiological role in vertebrate transmission. *Life Sci.* 21: 23-42.
332. Terenius, L., Wahlstrom, A., Lindstrom, L. & Widerlov, E. (1976). Increased CSF levels of endorphins in chronic psychosis. *Neuroscience Letters* 3: 157-162.
333. Akil, H., Madden, J., Patrick, R.L. & Barchas, J.D. (1976). In: Opiates and Endogenous Opioid Peptides, ed. Kosterlitz, H.W., pp. 63-70, North Holland Press, Amsterdam.
334. Bradley, P.B., Briggs, I., Gayton, R.J. & Lambert, L.A. (1976). Effects of microiontophoretically applied methionine-enkephalin on single neurones in rat brainstem. *Nature* 261: 425-426.
335. Bramwell, G.J. & Bradley, P.B. (1974). Actions and interactions of narcotic agonists and antagonists on brain stem neurones. *Brain Res* 73: 167-170.
336. Gent, J.P. & Wolstencroft, J.H. (1976). Effects of methionine-enkephalin and leucine-enkephalin compared with those of morphine on brainstem neurones in cat. *Nature* 261: 426.

337. Hill, R.G., Pepper, C.M. & Mitchell, J.F. (1976). Depression of nociceptive and other neurones in the brain by iontophoretically applied met-enkephalin. *Nature* 262: 604-606.
338. Zieglgansberger, W., Fry, J.P., Herz, A., Moroder, L. & Wunsch, E. (1976). Enkephalin-induced inhibition of cortical neurones in rat and the lack of this effect in morphine tolerant dependent rats. *Brain Res.* 115: 160-164.
339. Zieglgansberger, W., & Fry, J.P. (1976). Actions of enkephalin on cortical and striatal neurones of naive and morphine tolerant/dependent rats. In: Opiates and Endogenous Opioid Peptides, ed. Kosterlitz, H.W. pp. 231-238, North Holland Press, Amsterdam.
340. Frederickson, R.C.A. & Norris, F.H. (1976). Enkephalin-induced depression of single neurons in brain areas with opiate receptors-antagonism by naloxone. *Science* 194: 440-442.
341. Davies, J. & Dray, A. (1976). Effects of enkephalin and morphine on Renshaw cells in feline spinal cord. *Nature* 262: 603-604.
342. Snyder, S.H. & Malthysse, S. (1975). Opiate receptor mechanisms. *Neurosciences Res. Prog. Bull.* 13.
343. Braude, M.C., Harris, L.S., May, E.L., Smith, J.P. & Villareal, J.E. (editors) (1974). Narcotic Antagonists. Raven Press, New York.
344. Kosterlitz, H.W., Collier, H.O.J. & Villarreal, J.E. (editors) (1973). Agonist and Antagonist Actions of Narcotic Analgesic Drugs. Park Press, Baltimore.
345. Ford, D.H. & Clouet, D.H. (1976). Tissue Responses to Addictive Drugs. Spectrum Publications, New York.
346. Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E. & Gilbert, P.E. (1976). The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J. Pharmacol. exp. Ther.*
347. Hutchinson, M., Kosterlitz, H.W., Leslie, F.M., Waterfield, A.A. & Terenius, L. (1975). Assessment in the guinea-pig ileum and mouse vas deferens of benzomorphans which have strong antinociceptive activity but do not substitute for morphine in the dependent monkey. *Brit. J. Pharmacol.* 55: 541-546.
348. Lord, J.A.H., Waterfield, A.A., Hughes, J. & Kosterlitz, H.W. (1977). Endogenous opioid peptides: multiple agonist and receptors. *Nature* 267: 495-499.

349. Puig, M., Gascon, P., Craviso, G. & Musacchio, J.M. (1977). Endogenous opiate receptor ligand: electrically induced release in the guinea pig ileum. *Science* 195: 419-420.
350. Abood, L.G. & Hoss, W. (1975). Stereospecific morphine adsorption to phosphatidyl serine and other membranes components of brain. *Eur. J. Pharmacol.* 32: 66-75.
351. Bird, S.J. & Kuhar, M.J. (1977). Iontophoretic application of opiates to the locus coeruleus. *Brain Res.* 122: 523-533.
352. Nicoll, R.A., Siggins, G.R., Ling, N., Bloom, F.E. & Guillemin, R. (1977). Neuronal actions of endorphins among brain regions: a comparative microiontophoretic study. *Proc. Natl. Acad. Sci. USA* 74: 2584-2588.
353. Duggan, A.W., Hall, J.G. & Headley, P.M. (1976). Morphine, enkephalin and the substantia gelatinosa. *Nature* 264: 456-458.
354. McCarthy, P.S., Walker, R.J. & Woodruff, G.N. (1976). Depressant actions of enkephalins on neurones in the nucleus accumbens. *Proc. Soc. J. Physiol.* 40P.
355. Andersen, R.K., Lund, J.P. & Puil, E. (Unpublished). Enkephalin and substance P effects related to trigeminal pain. 1977.
356. Dostrovsky, J. & Pomeranz, B. (1973). Morphine blockade of amino acid putative transmitters on cat spinal cord sensory interneurons. *Nature* 246: 222-224.
357. Davies, J. & Dray, A. (1977). Substance P and opiate receptors. *Nature* 268: 351-352.
358. Ritchie, J.M. & Armet, C.J. (1963). On the role of acetylcholine in conduction in the mammalian nonmyelinated nerve fibres. *J. Pharmac. exp. Ther.* 139: 201-207.
359. Frazier, D.T., Ohta, M. & Narahashi, T. (1973). Nature of the morphine receptor present in the squid axon. *Proc. Soc. exp. Med.* 142: 1209-1214.
360. Galindo, A. (1971). Procaine, pentobarbital and halothane: effects on the mammalian myoneural junction. *J. Pharmacol. exp. Ther.* 177: 360-368.
361. Galindo, A. (1972). Anesthesia and synaptic transmission. In: *Cellular Biology and Toxicity of Anesthetics*, ed. Fink, B.R., pp. 69-76, Williams & Wilkins, Baltimore.
362. Waterfield, A.A., Smokcum, R.W.J., Hughes, J., Kosterlitz, H.W. & Henderson G. (1977). In vitro pharmacology of the opioid peptides, enkephalins and endorphins. *Eur. J. Pharmacol.* 43: 107-116.

363. North, R.A. & Henderson, G. (1975). Action of morphine on guinea-pig myenteric plexus and mouse vas deferens studied by intracellular recording. *Life Sci.* 17: 63-66.
364. North, R.A. & Tonini, M. (1976). Hyperpolarization by morphine of myenteric neurones. In: *Opiates and Endogenous Opioid Peptides*, ed. Kosterlitz, H.W. pp. 205-212, North Holland Press, Amsterdam.
365. North, R.A. (1976). Effects of morphine on myenteric plexus neurones. *Neuropharmacol.* 15: 719-721.
366. North, R.A. & Williams, J.T. (1976). Enkephalin inhibits firing of myenteric neurones. *Nature* 264: 460-461.
367. North, R.A. (1977). Hyperpolarization of myenteric neurones by enkephalin. *Proc. B.P.S.*, Jan. 505P.
368. Henderson, G. & Hughes, J. (1976). The effects of morphine on the release of noradrenaline from the mouse vas deferens. *Br. J. Pharmac.* 57: 551-557.
369. Henderson, G. & North, R.A. (1976). Depression by morphine of excitatory junction potentials in the vas deferens of the mouse. *Br. J. Pharmac.* 57: 341-346.
370. Frederickson, R.C.A. & Pinsky, C. (1971). Morphine impairs acetylcholine release but facilitates acetylcholine action at a skeletal neuromuscular junction. *Nature New Biol.* 231: 93-94.
371. Pinsky, C. & Frederickson, R.C.A. (1971). Morphine and nalorphine impair neuromuscular transmission. *Nature New Biol.* 231: 94-96.
372. Bell, K.M. & Rees, J.M.H. (1974). The depressant action of morphine on transmission at a skeletal neuromuscular junction is non-specific. *J. Pharm. Pharmac.* 26: 686-691.
373. Buttar, H.S. & Frank, G.B. (1977). Effects of antipsychotic drugs on action potential production in skeletal muscle. I. Chlorpromazine and promethazine. *Can. J. Physiol. Pharmacol.* 55: 452-461.
374. Jhamandas, K., Phillis, J.W. & Pinsky, C. (1971). Effects of narcotic analgesics and antagonists on the *in vivo* release of acetylcholine from the cerebral cortex of the cat. *Br. J. Pharmac.* 43: 53-66.
375. Durham, H.D., Frank, G.B. & Marwaha, J. (1977). Effects of anti-psychotic drugs on action potential production in skeletal

muscle. II. Haloperidol: nonspecific and opiate drug receptor mediated effects. *Can. J. Physiol. Pharmacol.* 55: 462-470.

376. Zieglgansberger, W. & Bayerl, J. (1976). Interactions of morphine and atropine with putative transmitters. In: Drugs and Central Synaptic Transmission, eds. Bradley & Dhawan, pp. 131-138, Univ. Pk. Press.
377. Satoh, M., Zieglgansberger, W., Fries, W. & Herz, A. (1976). Effects of opiates and opiate antagonists on cortical neurones in naive and tolerant-dependent rats. In: Drugs and Central Synaptic Transmission, eds. Bradley & Dhawan, pp. 317-323, Univ. Pk. Press.
378. Narahashi, T. (1974). Chemicals as tools in the study of excitable membranes. *Physiological Rev.* 54: 813-889.
379. Whittaker, V.P. (1974). Membranes in synaptic function. *Hospital Practice*, April, pp. 111-119.
380. Halsey, M.J. & Wardley-Smith, B. (1975). Pressure reversal of narcosis produced by anaesthetics, narcotics and tranquilizers. *Nature* 257: 811-813.
381. Feldberg, W. & Smyth, D.G. (1976). The C-fragment of lipotropin a potent analgesic. *J. Physiol.* 260: 30-31.
382. Hodgkin, A.L. & Katz, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* 108: 37-77.
383. Goldfarb, J. & Hu, J.W. (1976). Enhancement of reflexes by naloxone in spinal cats. *Neuropharmacol* 15: 785-792.
384. North-Root, H., Martin, D.W. & Toliver, A.P. (1976). Evidence for nuclear sites of stereospecific opiate binding in neuroblastoma cells in continuous culture. *Physio. Chem. & Physics.* 8: 437-446.
385. Ford, D.H. & Clouet, D.H. (1976). Concluding Remarks. In: Tissue Responses to Addictive Drugs, eds. Ford, D.H. & Clouet, D.H. pp. 679-693, Spectrum Publications, New York.
386. Yang, H.-Y., Hong, J.S. & Costa, E. (1977). Regional distribution of leu and met enkephalin in rat brain. *Neuropharmacol.* 16: 303-307.
387. Mitznegg, P., Domschke, W., Sprugel, W., Domschke, S., Subramanian, N., Wunsch, E., Moroder, L. & Demling, L. (1977). Enkephalins inhibit intestinal motility: mode of action. *Acta Hepato-Gastroenterol* 24: 119-120.

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